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**Synthesis of isocyanide derived natural products with antibiotic activity**

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# Synthesis of isocyanide derived natural products with antibiotic activity

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*A thesis submitted for the degree of doctor of philosophy*

*University of Bath*

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*August 2015*

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## Abstract

This thesis concerns itself with developing methodology for the synthesis of vinyl-isocyanide natural products and evaluating the biological properties of their common aglycone's vinyl-isocyanide warhead.

Firstly, methodology for the total synthesis of isocyanide containing natural products isolated from terrestrial and marine sources is reviewed, describing the different strategies that have been employed for their synthesis as well as any associated biological activity.

The development of a novel isocyanide derived phosphonate ester and its use in an *E*-selective Horner-Wadsworth-Emmons protocol for the synthesis of the aglycone, phenol vinyl-isocyanide, from *p*-hydroxybenzaldehyde is then described. It was found that increasing the steric bulk of phosphonate ester groups increased the *E*-selectivity of homologation from 7 : 3 to 9 : 1. Total selectivity was subsequently achieved when temporarily reducing the acidity of the substrates through phenol silylation, although these species typically began to isomerise upon desilylation and isolated samples of phenol vinyl-isocyanide typically possessed >95 d.e. Phenol vinyl-isocyanide is then demonstrated as a potent melanogenesis and bacterial inhibitor with low toxicity, leading us to develop some antibiotic structure activity relationships.

Having synthesised the aglycone, we intended to use this in the total synthesis of Rhabduscin and Byelyankacin. Being an unstable species, methodology was then developed for the synthesis of these rhamnopyranosides using *p*-hydroxybenzaldehyde for the glycosylation step. The previously developed Horner-Wadsworth-Emmons reagent was then used to achieve the first synthesis of Byelyankacin. Work is on-going towards completing the synthesis of a suitable rhamnopyranoside aldehyde for the synthesis of Rhabduscin using the same synthetic operations reported for Byelyankacin.

Finally, we report the synthesis of Paerucumarin, an isocyanide derived di-hydroxy coumarin species that has a related biosynthesis to Byelyankacin, Rhabduscin and their common glycone. This synthesis proceeds *via* the structurally related natural product, Pseudoverdin.

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## Abbreviations

AFA	acetic formic anhydride
approx.	approximately
b.p.	boiling point
Bn	benzyl
br.	broad
Bu	Butyl
CDMT	2-Chloro-4,6-bis[3-(perfluorohexyl)propyloxy]-1,3,5-triazine
CPBA	chloroperbenzoic acid
CSA	camphor sulfonic acid
d	doublet
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dd	doublet of doublets
ddd	doublet of doublets of doublets
delta	chemical shift
DIBAL	diisobutylaluminum hydride
DIEA	<i>N,N</i> -Diisopropylethylamine
DIPEA	diisopropyl amine
DMAP	dimethyl aminopyridine
DMAP	dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DMT	dimethoxytriphenylmethyl
e.g.	for example
EC50	half maximal effective concentration
equiv.	equivalent
Et	ethyl
Fig.	figure
g	gram
h.	hour
HMPA	hexamethylphosphoramide
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
<i>i</i>	iso
IBX	2-Iodoxybenzoic acid
IC50	half maximal inhibitory concentration
inc	including
IR	infra red
IUPAC	international union of pure and applied chemistry
<i>J</i>	coupling constant
KHMDS	potassium hexamethyldisilazane
LDA	lithium diisopropylamine
LHMDS	lithium hexamethyldisilazane
Ltd.	limited

<i>m</i>	meta
M	Mega
m	multiplet
m.p.	melting point
Me	methyl
mg	milligram
MIC	minium inhibitory concentration
min.	minute
mL	millilitre
mM	millimolar
MOM	methoxymethyl
MRSA	methicillin resistant staphylococcus aureus
MS	Mass Spec
Ms	Mesyl
NBS	<i>N</i> -bromosuccinamide
NCS	isothiocyanate
ng	nano gram
NHMDS	sodium hexamethyldisilazane
NIS	<i>N</i> -iodosuccinimide
NMR	nuclear magnetic resonance
<i>o</i>	ortho
<i>p</i>	para
PCC	pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	phenyl
PhosMIC	methyl isocyanophosphonate
ppm	parts per million
Pr	propyl
Py.	pyridine
q	quartet
quant.	quantative (yield)
r.t.	room temperature
SAR	structure activity relationships
t	triplet
<i>t</i>	tertiary
TBAF	tetra-butyl amoniumfluoride
TBS	tertbutly(dimethyl)silyl
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethyl silane
Tol.	toluene
Ts	tosyl
µg	micro gram
µM	micromolar

# **1 Literature Review – Methodology for the synthesis of isocyanide containing natural products (covering up to end of 2014)**

## **1.1 Introduction**

Naturally occurring isocyanides (or isonitriles) represent a diverse range of secondary metabolites that have been isolated from a select number of marine (Figure 1), terrestrial and freshwater (Figure 2) organisms, including bacteria, fungi, blue-green algae, plants, marine sponges and nudibranches.<sup>1</sup> These naturally occurring isocyanides have been shown to exhibit an impressive range of biological activity, with different classes of natural product known to exhibit antibiotic, antifungal, antiviral, anticancer, herbicidal, antifouling and antimalarial activity.<sup>2</sup>

Figure 1 Range of marine organisms from which isocyanide containing natural products have been isolated: **A** Sponge - *Axinella cannabina*; **B** Sponge - *Acanthella* sp.; **C** Sponge - *Hymeniacidon amphilecta*; **D** Nudibranch - *Phyllidia varicose* sp.

Figure 2 Range of terrestrial and freshwater bacteria and fungi responsible for the production of isocyanide containing natural products: **A** Cyanobacteria - *Hapalosiphon fontinalis*; **B** Cyanobacteria - *Fischerella* sp; **C** Fungi - *Aspergillus* sp.; **D** Fungi - *Trichoderma hamatum*.

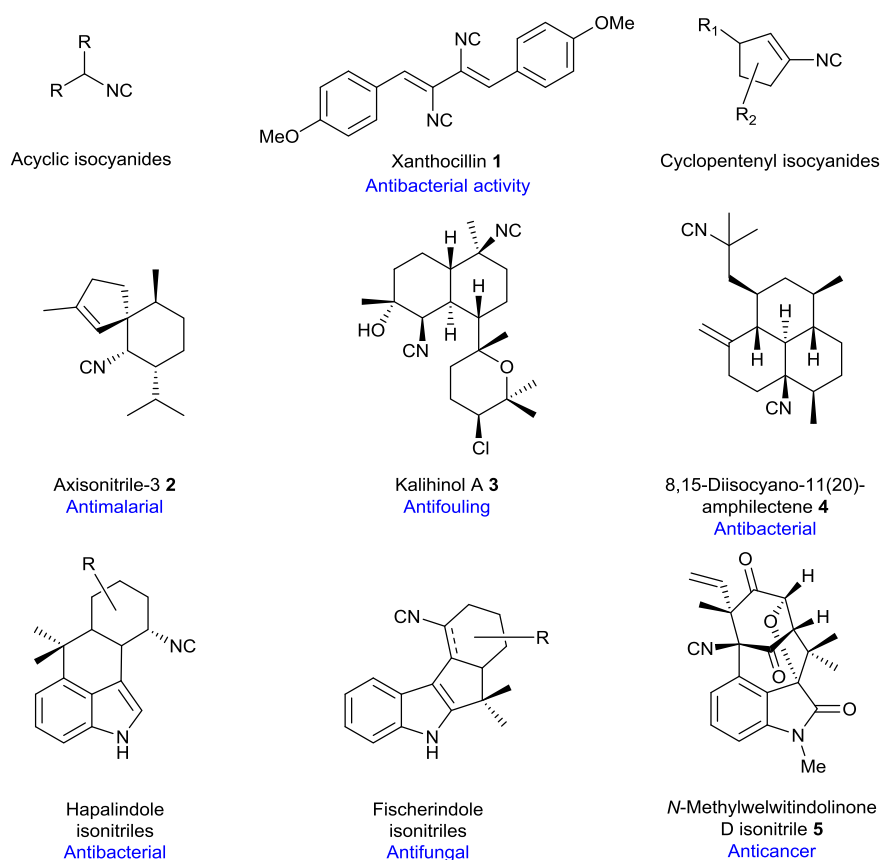


Figure 3 Representative range of isocyanide containing natural products that have been shown to exhibit potentially useful biological and medicinal activity.<sup>2</sup>

The structures of this fascinating class of natural product range from simple acyclic isocyanides through to complex polycyclic isocyanides that contain multiple stereocenters, many of which exhibit useful biological and medicinal properties (Figure 3). A number of these isocyanide natural products are highly toxic and are often produced as a defence mechanism by the host organism to deter predators. They normally emit a 'foul' and 'penetrating' odour, which often affords the first clue that a natural product containing an isocyanide group has been isolated. The existence of a strong stretching absorption band in the infrared spectra at around  $2100\text{ cm}^{-1}$  is also highly diagnostic for the presence of an isocyanide group.<sup>3</sup> Isocyanides are isomeric to their corresponding nitriles, containing an unusually stable trivalent mono-coordinated carbon atom, whose structure is best represented as a zwitterionic species that can potentially display nucleophilic, electrophilic and carbene-like character (Figure 4).<sup>4</sup> Consequently, it is unsurprising that many isocyanide containing natural products are relatively unstable, with their isocyanide functionalities having the capacity to react *via* a wide range of nucleophilic, electrophilic or oxidative pathways in biological systems.

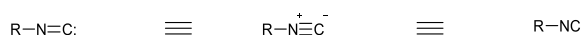


Figure 4 Structural representations of the isocyanide functional group.

Since the discovery of the first natural isocyanide xanthocillin **1** in 1957,<sup>5</sup> a growing number of naturally occurring isocyanide containing natural products have been isolated from fungal, bacterial and marine organisms. The biosynthetic origin of the isocyanide group in a number of these natural products has been investigated, with their biogenesis found to be dependent on whether they originate from marine or terrestrial organisms. Marine isocyanides, derived from either sesquiterpenoid or diterpene skeletons, are generally considered to be formed from reaction of inorganic cyanide with reactive cationic or epoxy terpenoid intermediates (Figure 5),<sup>6,7</sup> whilst terrestrial isocyanides are derived from functionalisation of amino acid precursors.<sup>2,7</sup> For example, elegant isotopic labelling studies ( $^{13}\text{C}$  and  $^{14}\text{C}$ ) have demonstrated that inorganic cyanide is incorporated into terpenoid isocyanides originating from a range of different sponges,<sup>8-11</sup> as shown for the proposed biosynthetic pathways leading to axisonitrile-3 **2** (Figure 6),<sup>12,13</sup> and the amphilectane isocyanides **9** and **10** (Figure 7).<sup>5,14</sup> Current evidence suggests that these terpenoid isocyanides are formed as direct metabolic products of the sponges themselves, rather than being produced by symbiotic cyanobacteria,<sup>6</sup> with these reactive metabolites thought to be produced for their antifeedant, antifouling and/or toxic properties. Isocyanides have also been shown to be present in some species of nudibranch,



however these isocyanides are thought to accumulate by dietary transfer from sponge feeding.<sup>15</sup>

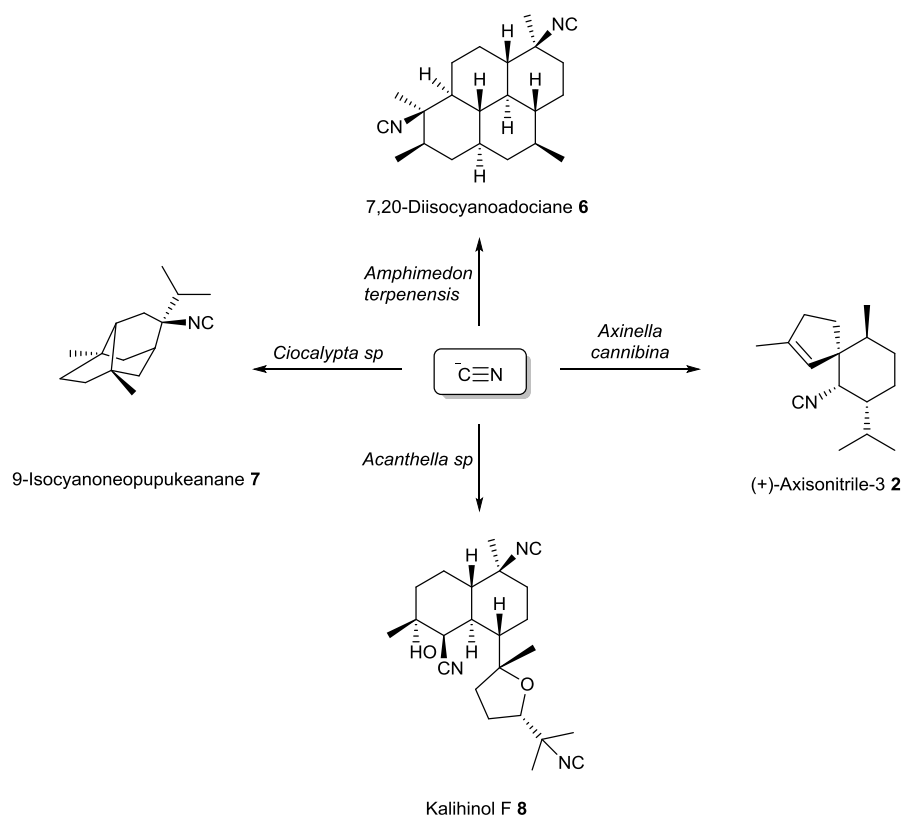


Figure 5 The isocyanide groups of marine terpenoid natural products **2,6-8** are derived from reaction of inorganic cyanide with reactive cationic intermediates generated during terpene biosynthesis.<sup>7,8,10,12</sup>

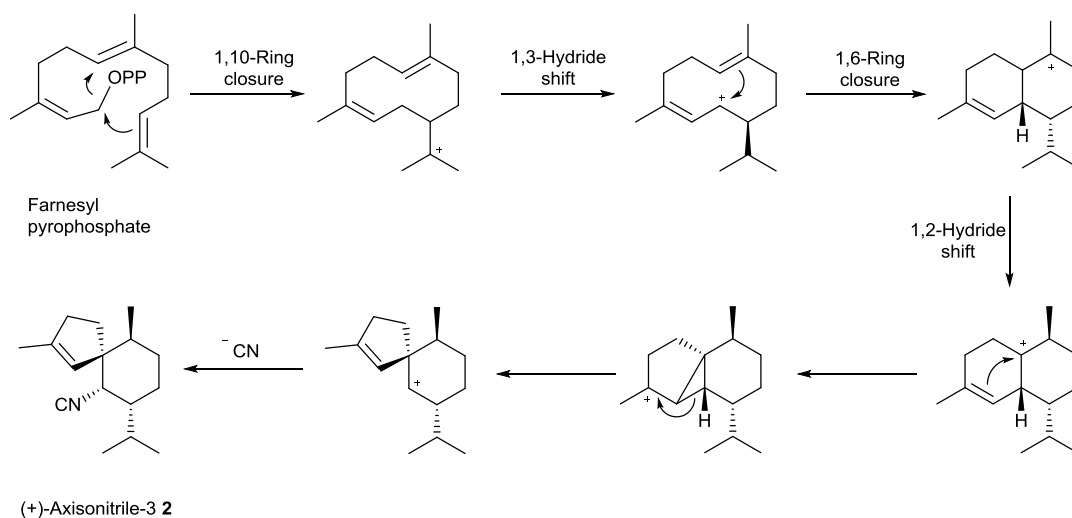


Figure 6 Proposed biosynthetic pathway leading to axisonitrile-3 **2**.<sup>12,13</sup>

## Methodology for the synthesis of isocyanide containing natural products

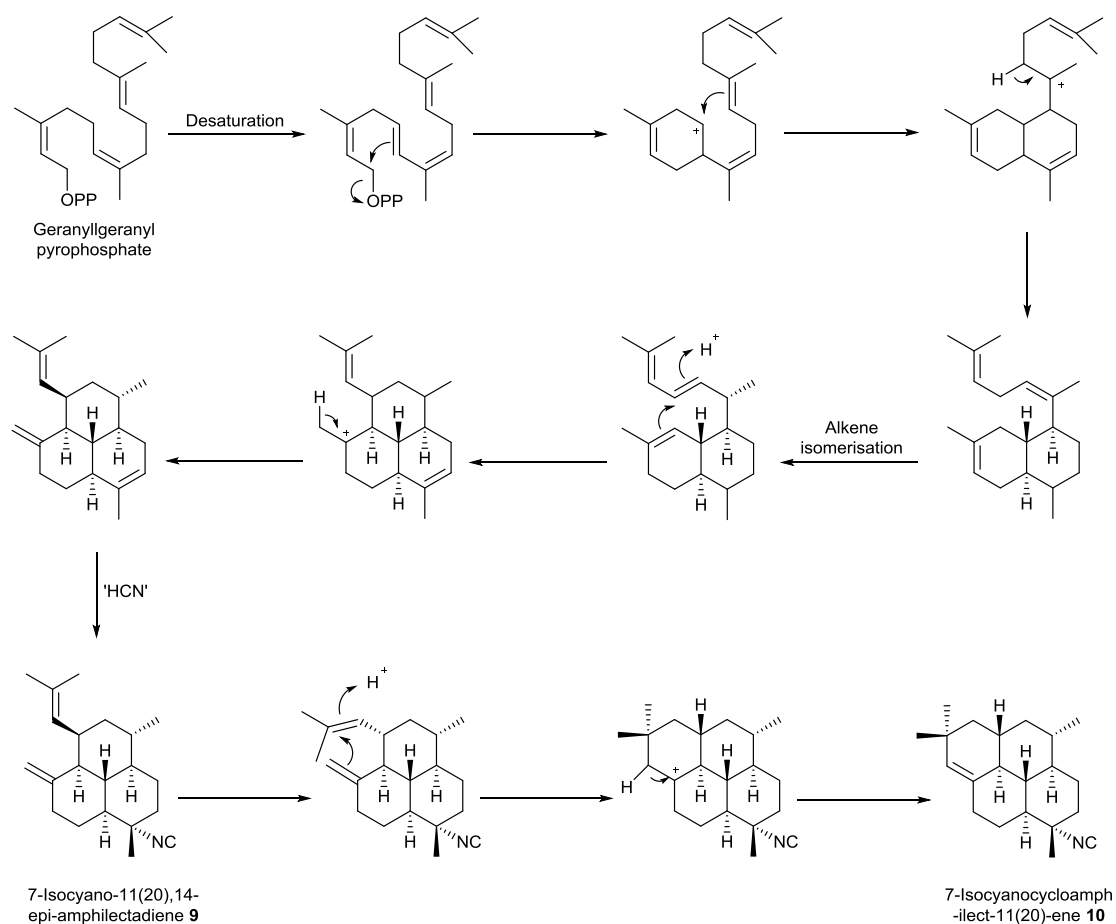


Figure 7 Proposed biosynthetic pathways leading to tricyclic and tetracyclic amphilectane diterpene isocyanides **9** and **10**.<sup>6,14</sup>

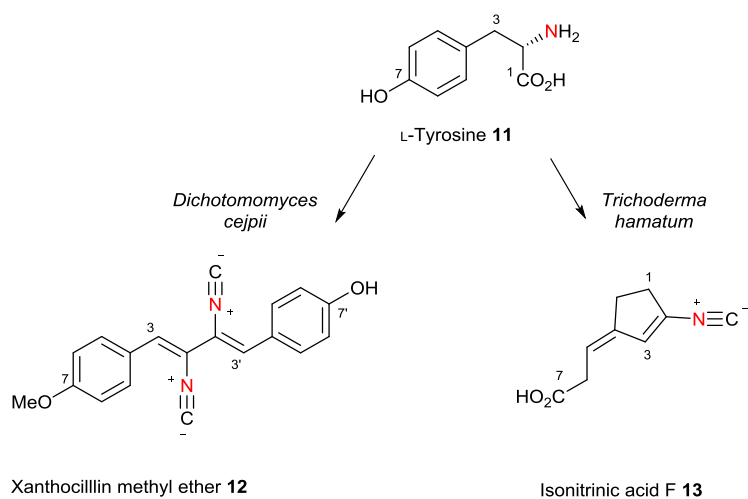


Figure 8 Biosynthesis of L-tyrosine derived isocyanide natural products **12**, **13**.<sup>16,17</sup>

Many terrestrial isocyanide natural products have been shown to be derived from selective functionalisation of the skeletons of  $\alpha$ -amino acid precursors such as L-tyrosine or L-tryptophan, with the isocyanide functionalities of these natural products retaining the nitrogen atom of the parent amino acid (Figure 8). For example, Herbert and co-workers have shown that the nitrogen atoms of xanthocillin monomethyl ether **12** are derived from the amino groups of L-tyrosine **11** in *Dichotomomyces cejpai*,<sup>16</sup> whilst Baldwin and co-workers have demonstrated that *Trichoderma hamatum* converts the *p*-hydroxybenzyl side chain of L-tyrosine into the carbocyclic ring and acid side chain of isonitrinic acid **13**.<sup>17</sup> The origin of the carbon atom of the isocyanide fragment in the majority of terrestrial natural products remains unknown, although isotope labelling studies in the terrestrial cyanophyte *Hapalosiphon fontinalis* suggested that the isocyanide carbon in hapalindole A isonitrile originated from a C<sub>1</sub>-donor originating from tetrahydrofolate metabolism.<sup>18</sup> However, investigations by Clardy and Brady into bacterial metabolic pathways have shown that the carbon atom of indole isocyanide **17** originates from the C<sub>2</sub>-sugar atom of ribulose-5-phosphate **15**.<sup>19</sup> In this case, the enzyme *IsnA* was shown to be responsible for converting the amino group of L-tryptophan **14** into the isocyanide functionality of  $\alpha$ -isocyano acid **16**, with another enzyme *IsnB* then catalysing its decarboxylation to introduce the vinyl functionality of indole isocyanide **17** (Figure 9).<sup>20</sup> A similar pathway has been proposed for the biosynthesis of paerucumarin **19** in *Pseudomonas aeruginosa*, with *PvcA* and *PvcB* being responsible for formation of isocyanide intermediate **18** that is then oxidatively transformed into the natural product *via* the action of *PvcA* and *PvcD* (Figure 9).<sup>21</sup>

Alternatively, structurally complex polycyclic monoterpene indole alkaloid isocyanides are believed to originate from chloronium induced cyclisation of a common indole (*Z*)-vinyl-isocyanide intermediate **20** onto a terpene derived triene precursor **21**. The isopropenyl fragment of 12-*epi*-hapalindole E isonitrile **23** then affords a carbocation intermediate that can undergo selective cyclisation onto different positions of its indole core to afford the respective skeletons of the hapalindoles, fischerindoles, welwitindolinones and ambiguine families of isocyanide natural products (Figure 10).<sup>22</sup>

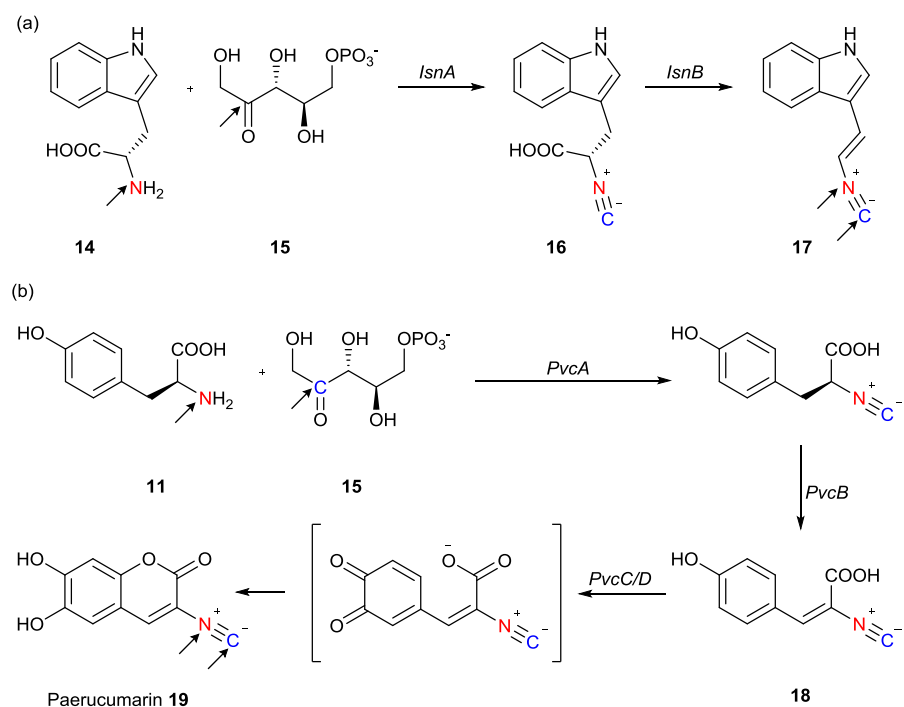


Figure 9 Biosynthetic origins of the carbon and nitrogen atoms found in the terrestrial vinyl isocyanides:  
(a) antibiotic isocyanide **17** and (b) paerucumarin **19**.<sup>19–21</sup>

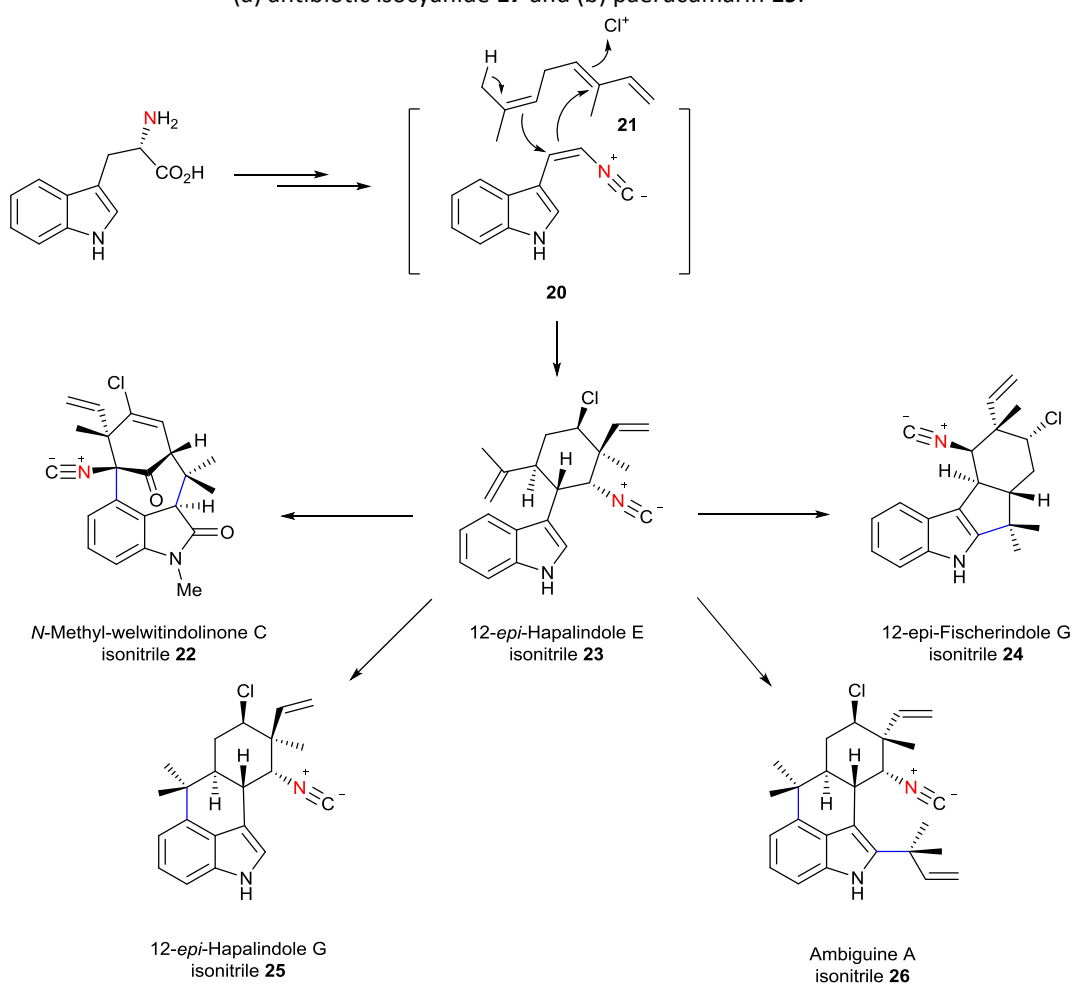


Figure 10 Biosynthesis of L-tryptophan derived monoterpenoid indole alkaloid isocyanides **22–26**.<sup>22</sup>

The metabolic fate of most isocyanide natural products remains unknown, however Kobayashi and co-workers have demonstrated the presence of isocyanide hydratases ( $R-N\equiv C \rightarrow R-NHCHO$ ) in *Pseudomonas putida* N19-2<sup>23</sup> and *Athrobacter pascens* F164,<sup>24</sup> and the occurrence of an *N*-substituted formamide deformylase ( $R-NHCHO \rightarrow R-NH_2$ ) in *Athrobacter pascens* F164,<sup>24</sup> which gives some insight into the hydrolytic pathways used for their metabolism/degradation. Enzymatic catalysed isocyanide hydration in *Pseudomonas fluorescens* is thought to occur *via* nucleophilic attack of the thiol group of a cysteine functionality at the isocyanide carbon within its active site, followed by aspartic acid mediated hydrolysis of the resultant enzyme bound thioimide intermediate by an incipient water molecule, followed by tautomerisation to afford a formamide product (Figure 11).<sup>25</sup>

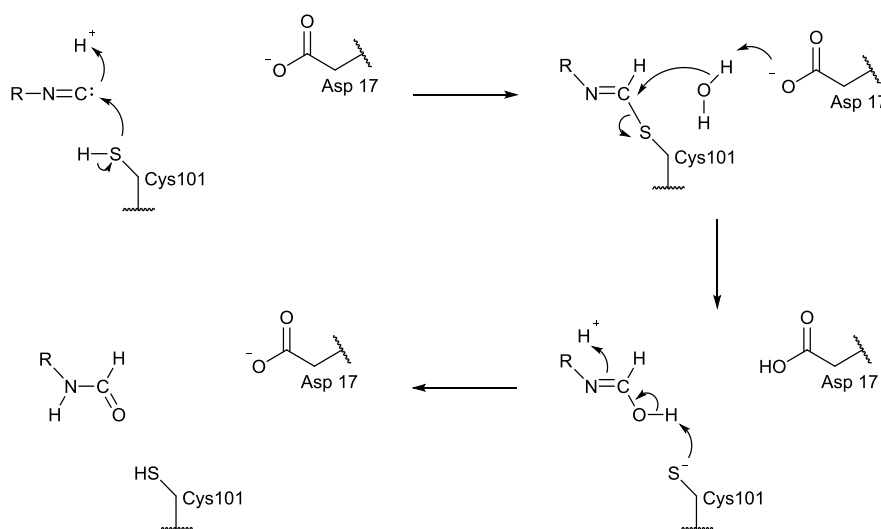


Figure 11 Mechanism of *Pseudomonas fluorescens* isocyanide hydratase catalysed hydration of an isocyanide to afford its corresponding formamide.<sup>25</sup>

Many of these fascinating isocyanide containing natural products are only available in relatively small amounts from their natural sources, with the host organism often being difficult to obtain, or requiring specialised microbiological skills to culture. Because of this scarcity, significant efforts have been directed towards the development of methodology for their synthesis, with the ultimate aim of producing significant quantities of these natural products (and their structural analogues) so that their biological activities can be fully evaluated. However, the structural complexity and diversity of many of these naturally occurring isocyanides, coupled with difficulties in handling their relatively reactive isocyanide functionalities, means that their synthesis represents a significant challenge. A number of

reviews have been published describing the range of isocyanide containing natural products that have been isolated, categorising these natural products according to their origin, structure and associated biological activity.<sup>1,2,26</sup> However, despite a recent review on marine terpenoids derived from inorganic cyanide,<sup>27</sup> the last detailed review describing the synthesis of isocyanides was published in 1988,<sup>26</sup> since which time there have been significant developments in the methodology available for their synthesis. Consequently, this review now describes in detail the different strategies that have been developed to construct the different skeletons of this fascinating class of natural product, describing how their reactive isocyanide functionalities were introduced in each case.

## 1.2 Syntheses of marine isocyanides derived from sesquiterpene biosynthetic pathways

Sponges from the genus *Axinella*, *Acanthella*, *Halichondria* and *Coicalypta* have been shown to produce a range of sesquiterpene isocyanides, with over 35 different marine derived C<sub>16</sub> isocyanides having been reported to date, many of which show promising biological activities (Figure 12).<sup>28–30</sup> These naturally occurring sesquiterpenoid isocyanides belong to eight different skeletal types comprised of one or two cyclic ring systems that normally contain a single isocyanide group. These isocyanides are often isolated in the presence of their corresponding isothiocyanate and *N*-formamide analogues, and have also been shown to accumulate in nudibranchs species. Total syntheses of a range of bisabolene, axane, spiroaxane, guaiane, pupukeanane, trachyopsane and cadinene derived isocyanides have been reported to date.

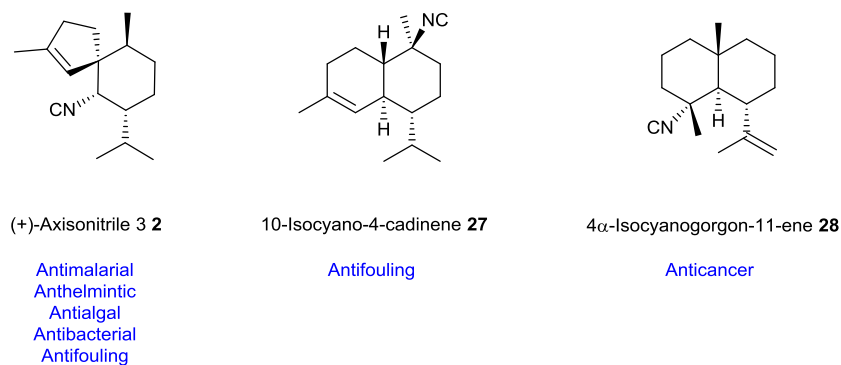
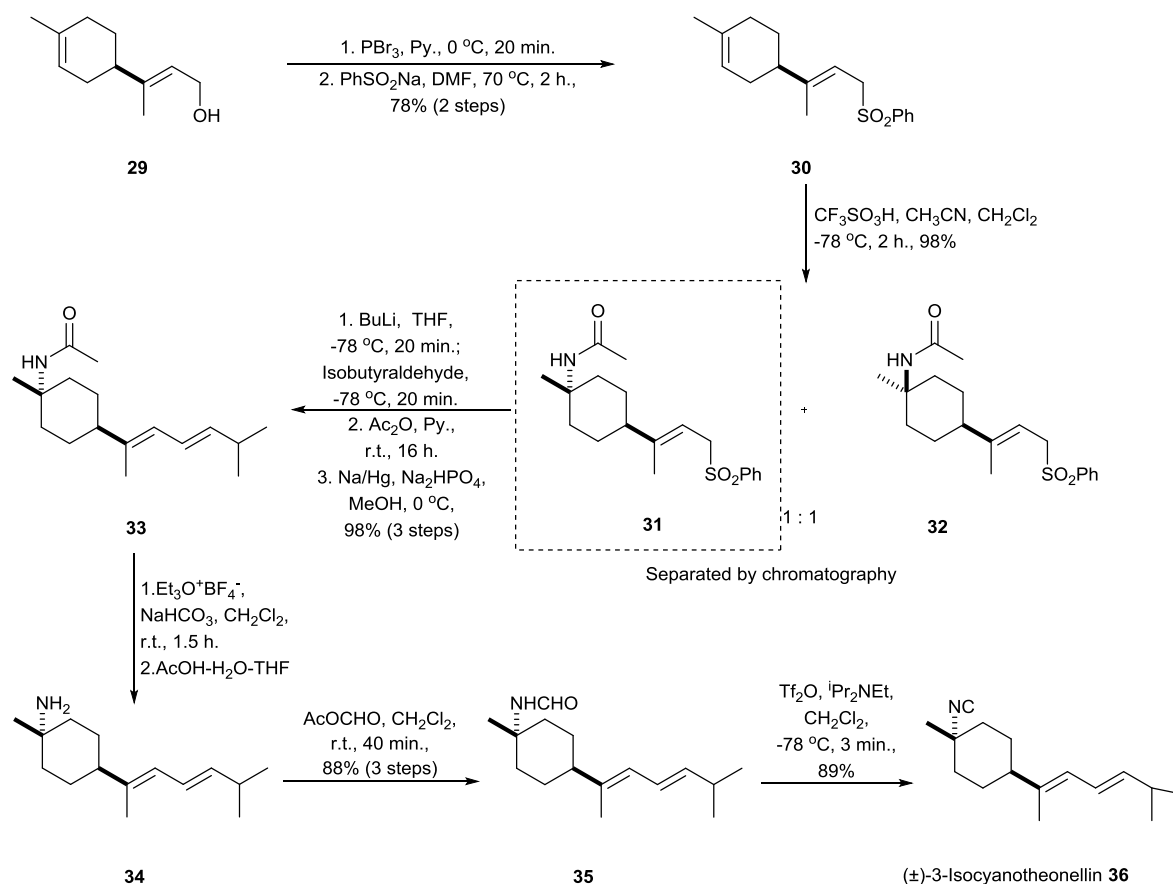


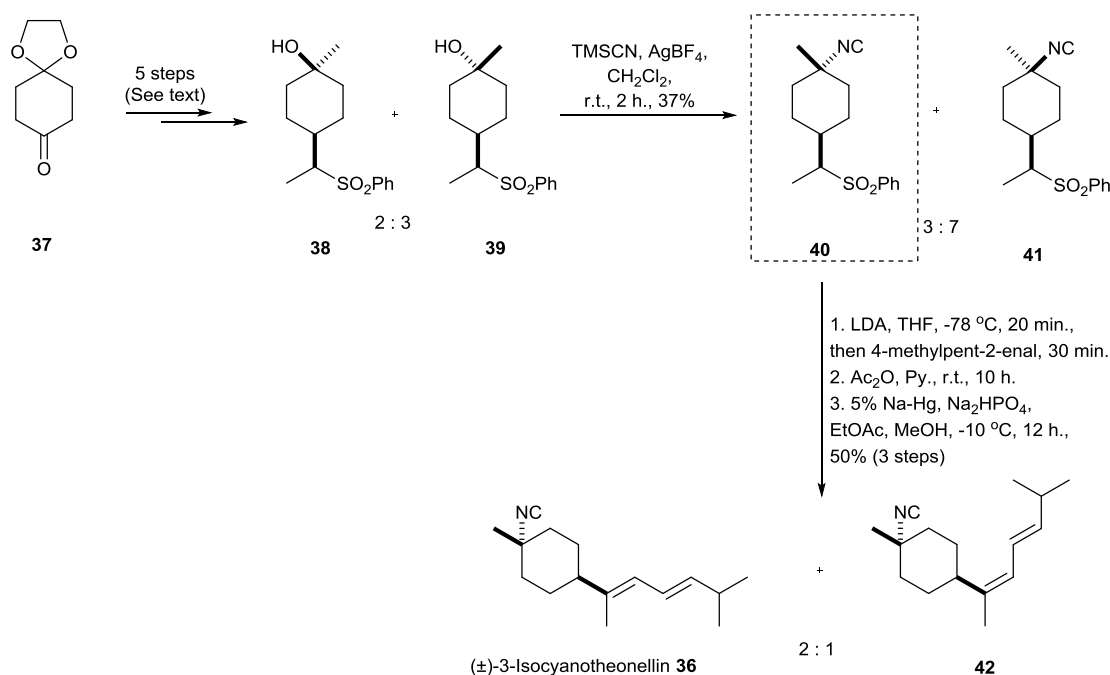
Figure 12 Biologically active sesquiterpene derived isocyanide natural products **2,27,28**.<sup>28–30</sup>

## 1.1.1. Syntheses of bisabolene derived isocyanides

Ichikawa reported the first synthesis of ( $\pm$ )-3-isocyanotheonellin **36** (Scheme 1),<sup>31</sup> a bisabolene sesquiterpene natural product that was isolated from extraction of the nudibranch *Phyllidia sp.* and shown to have anti-fouling activity against barnacle larvae.<sup>32</sup> For its synthesis, allyl alcohol **29** was treated with phosphorus tribromide, followed by nucleophilic displacement of the resultant bromide with sodium benzenesulfinate to afford allyl sulfone **30**, which on treatment with triflic acid underwent a Ritter reaction with acetonitrile to generate a 1 : 1 mixture of acetamides **31** and **32**. After chromatographic separation, isomer **31** underwent a Julia *trans*-olefination reaction with isobutyraldehyde to afford diene **33**, which was reacted with  $\text{Et}_3\text{O}^+\text{BF}_4^-$  and the resultant imino ether hydrolysed *in situ* with aqueous acetic acid to produce an amine **34** that was *N*-formylated using acetic formic anhydride to generate formamide **35**. Dehydration of formamide **35** with triflic anhydride in the presence of *N,N*-diisopropylethylamine (DIPEA) afforded ( $\pm$ )-3-isocyanotheonellin **36** that was spectroscopically identical to a natural sample.<sup>31</sup>

Scheme 1 Ichikawa's synthesis of ( $\pm$ )-3-isocyanotheonellin **36**.<sup>31</sup>

Zitano *et al.* have reported an alternative strategy employing silver cyanide as a nucleophile to intercept a tertiary cyclohexyl carbocation for the synthesis of ( $\pm$ )-3-isocyanotheonellin **36** and its geometric isomer **42** (Scheme 2).<sup>33</sup> A 2 : 3 mixture of tertiary alcohols **38** and **39** were first prepared in five steps from commercially available 1,4-cyclohexanedione mono-ethylene ketal **37** involving: (i) reduction of the ketone functionality; (ii) *O*-tosylation of the resultant secondary hydroxyl group; (iii) reaction of the tosylate with the lithium anion of ethyl phenyl sulfone; (iv) acid catalysed acetal deprotection; (v) reaction of the resultant ketone group with MeLi. Treatment of the resultant mixture of epimeric alcohols **38** and **39** with trimethylsilylcyanide (TMSCN) in the presence of AgBF<sub>4</sub> resulted in an S<sub>N</sub>1 reaction to give the desired isocyanides **40** and **41** in 92% yield, in an isomeric ratio of 3 : 7 in favour of the *trans*-isomer **41**. These isomeric isocyanides were separated by chromatography and the minor isocyanide **40** treated with 4-methylpent-2-enal under Julia conditions to afford a 2 : 1 mixture of ( $\pm$ )-3-isocyanotheonellin (*E,E*)-**36** and its geometric isomer (*E,Z*)-**42**, which could be separated by preparative reverse phase HPLC.<sup>33</sup>

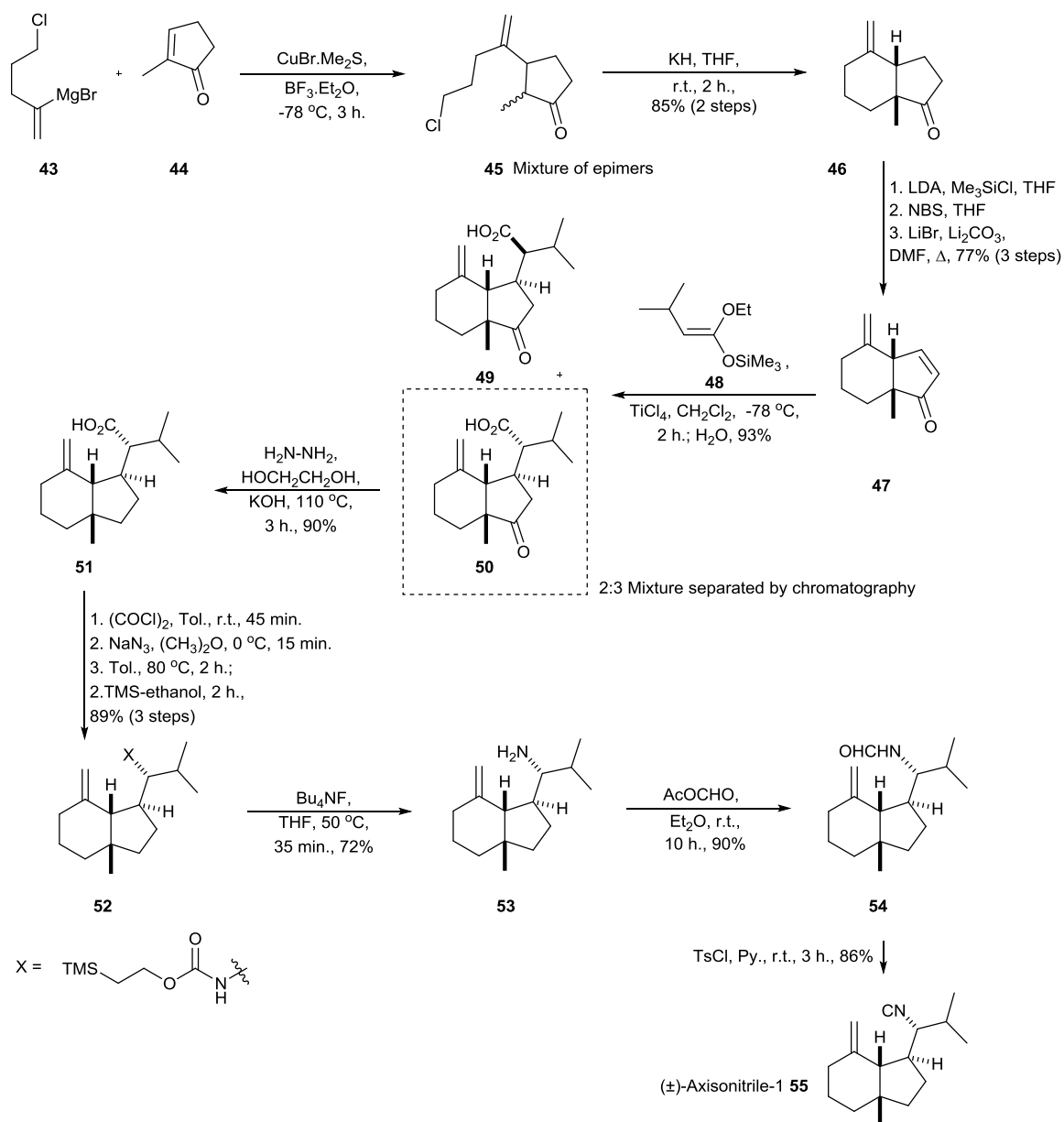


Scheme 2 Kitano's synthesis of ( $\pm$ )-(*E,E*)-3-isocyanotheonellin **36**.<sup>33</sup>



### 1.1.2. Syntheses of the axisonitriles

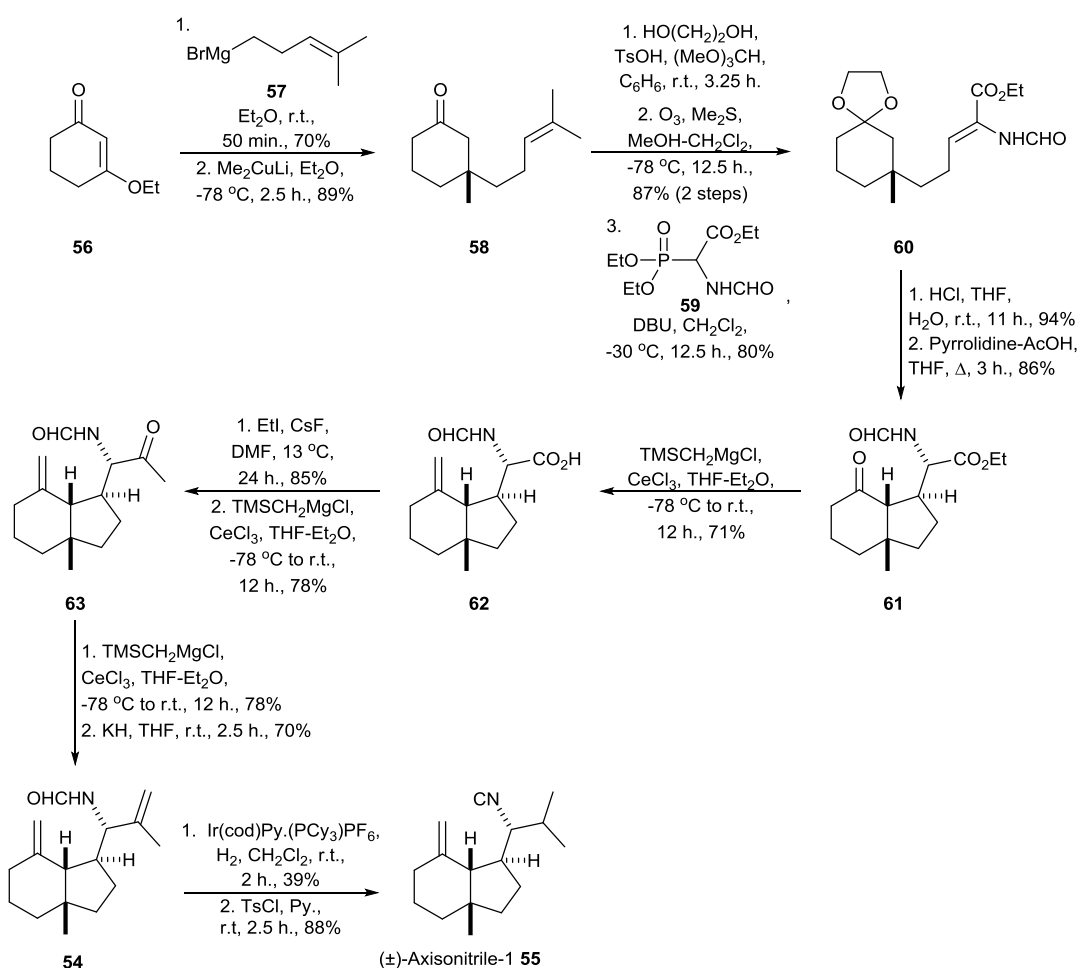
The axisonitriles are a class of sesquiterpenoid isocyanides that were isolated from chromatographic fractionation of the organic extracts of the marine sponge *Axinella cannabina*, that are thought to be a key component of the sponge's defence mechanism.<sup>13,34,35</sup> Piers *et al.* reported the first synthesis of (±)-axisonitrile-1 **55** in 1987 (Scheme 3),<sup>36,37</sup> which commenced with the copper catalysed conjugate addition of vinyl Grignard reagent **43** to cyclopenten-1-one **44** to generate ω-chloroketone **45** (as a mixture of epimers), whose potassium enolate then underwent an intramolecular alkylation reaction to afford bicyclic ketone **46** in 85% overall yield. Bicyclic ketone **46** was subsequently converted into its silyl enol ether, α-brominated and dehydrobrominated to produce enone **47**, which was treated with *bis*-silyl ketene acetal **48** in the presence of a catalytic amount of TiCl<sub>4</sub> under Mukaiyama conditions, resulting in an intermolecular conjugate addition reaction to afford an epimeric 2 : 3 mixture of γ-keto-acids **49** and **50**. This mixture of ketones **49/50** was separated by chromatography and γ-keto-acid **50** reduced under Wolf-Kishner conditions to afford carboxylic acid **51** that was converted into its corresponding acyl azide before undergoing a thermal Curtius rearrangement in the presence of 2-trimethylsilyl-ethan-1-ol to afford carbamate **52**. This carbamate **52** was then deprotected via treatment with tetrabutyl ammonium fluoride (TBAF) to afford amine **53**, which was *N*-formylated using acetic formic anhydride to give formamide **54** that could be dehydrated using *para*-toluenesulfonylchloride (TsCl) in pyridine to afford (±)-axisonitrile-1 **55**.<sup>36,37</sup>



Scheme 3 Piers' synthesis of (±)-axisonitrile-1 **55**.<sup>36,37</sup>

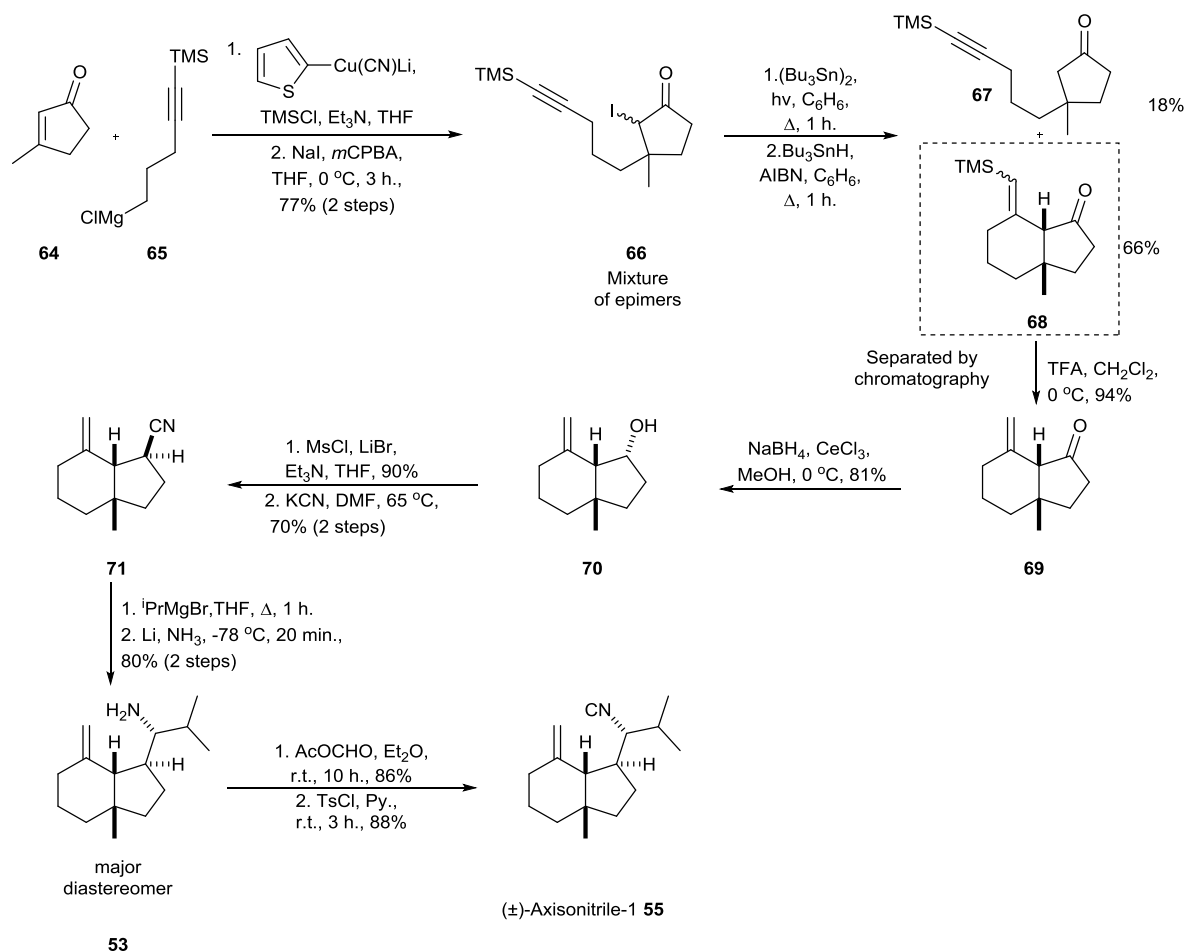
Hart and Guevel also reported a synthesis of (±)-axisonitrile-1 **55** that commenced with conjugate addition of an unsaturated cuprate reagent **57** to β-ethoxy-α,β-unsaturated ester **56**, with ethoxide elimination generating an α,β-unsaturated ester intermediate that underwent a further conjugate addition reaction with Me<sub>2</sub>CuLi to install the quaternary centre of ketone **58** (Scheme 4).<sup>38</sup> This ketone **58** was then protected as an acetal and its alkene bond ozonised to afford an aldehyde intermediate that was reacted with Horner-Wadsworth-Emmons reagent **59** to afford formamide **60**. The bicyclic [4.3.0] core of the natural product was then generated *via* acidic deprotection of acetal **60** to afford a ketone intermediate, which

was treated with pyrrolidine/acetic acid to generate an enamine intermediate that underwent a stereoselective intramolecular conjugate addition reaction to afford *cis*-fused bicyclic ester **61**. Ester **61** was then transformed into *bis*-alkene-formamide **54** (via acid **62** and ketone **63**) using a series of cerium catalysed methylenation reactions using TMSCH<sub>2</sub>MgCl as a nucleophile to introduce both of the *exo*-methylene fragments. Selective hydrogenation of the isopropylene alkene functionality of formamide **54** using Crabtree's catalyst (Ir(cod)py(PyCy)<sub>3</sub>PF<sub>6</sub>) was then followed by dehydration of the formamide group with TsCl in pyridine to give (±)-axisonitrile-1 **55**.<sup>38</sup>

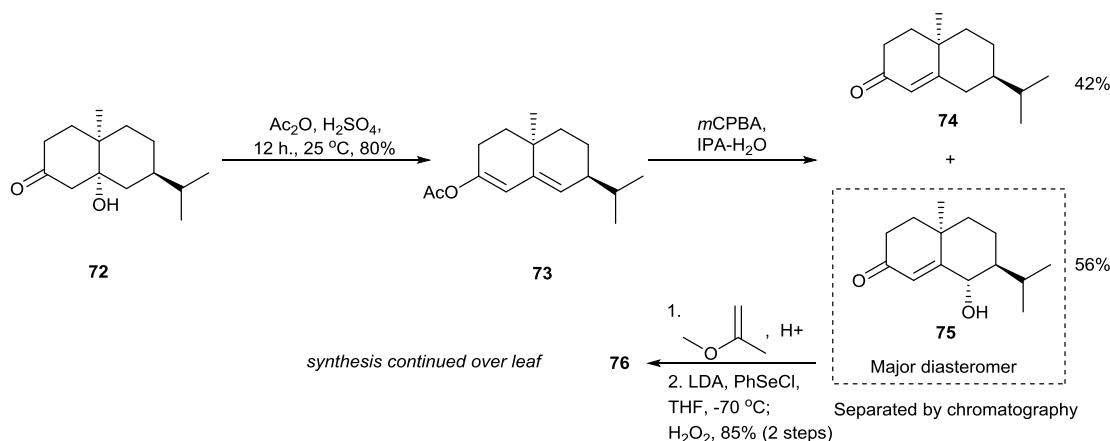
Scheme 4 Hart's synthesis of (±)-axisonitrile-1 **55**.<sup>38</sup>

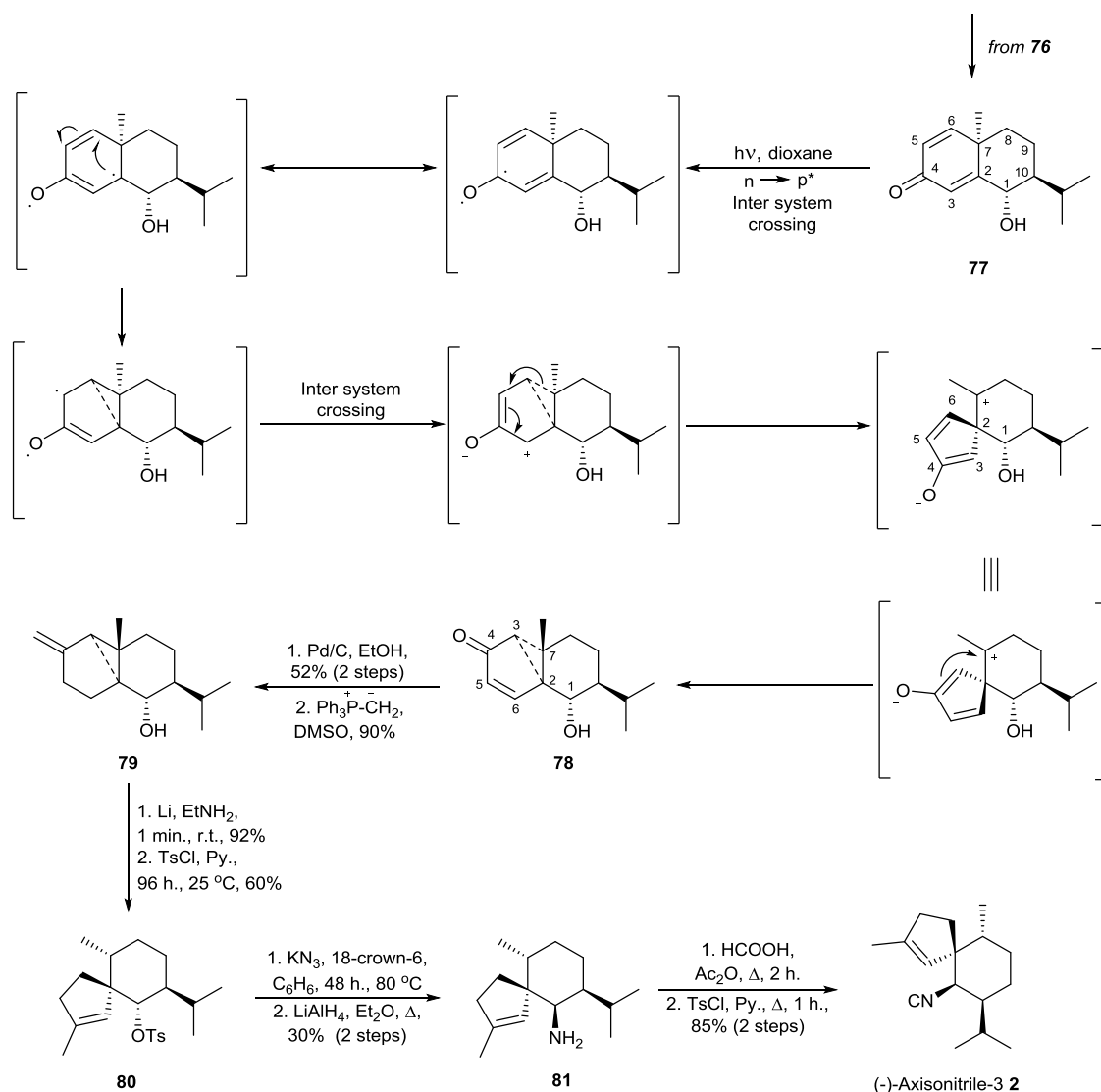
In 2008, Sha and co-workers reported an alternative synthesis of (±)-axisonitrile-1 **55** using a 6-*exo*-dig radical cyclisation approach to construct its bicyclic core (Scheme 5).<sup>39</sup> Conjugate addition of a cuprate derived from TMS-alkynyl Grignard reagent **65** to cyclopentenone **64** was followed by *in situ* trapping of the resulting enolate as a silyl enol ether, which was then treated with NaI and *meta*-chloroperoxybenzoic acid (*m*CPBA) to generate α-iodo ketone **66** (as a mixture of epimers). Irradiation of iodide **66** with light in the presence of hexabutylditin

generated a radical species that underwent an intramolecular 6-*endo*-dig cyclisation reaction, which was followed by Bu<sub>3</sub>SnH/AIBN mediated reduction to generate a mixture of bicyclic-[4.3.0]-vinyl-silanes (*Z*)-/(*E*)-**68**, as well as the unwanted reduced monocyclic ketone **67**. After purification by chromatography the mixture of (*Z*)/(*E*)-ketones **68** was treated with trifluoroacetic acid (TFA), which resulted in protodesilylation into ketone **69** that underwent stereoselective reduction with NaBH<sub>4</sub>/CeCl<sub>3</sub> to afford *endo*-alcohol **70**. Mesylation of alcohol **70** was followed by reaction with cyanide anion resulting in clean S<sub>N</sub>2 inversion to afford nitrile **71**, which was then treated with <sup>i</sup>PrMgBr to afford an imine intermediate that was reduced with Li/NH<sub>3</sub> to afford amine **53**. (±)-Axisonitrile-1 **55** was then generated *via* formylation of amine **53** with acetic formic anhydride, followed by dehydration of the resultant formamide using TsCl in pyridine.<sup>39</sup> Honda and co-workers subsequently published an alternative synthesis of the advanced ketone intermediate **69**, enabling them to claim a formal synthesis of (±)-Axisonitrile-1 **55**.<sup>40</sup>


 Scheme 5 Sha's synthesis of (±)-axisonitrile-1 **55**.<sup>39</sup>

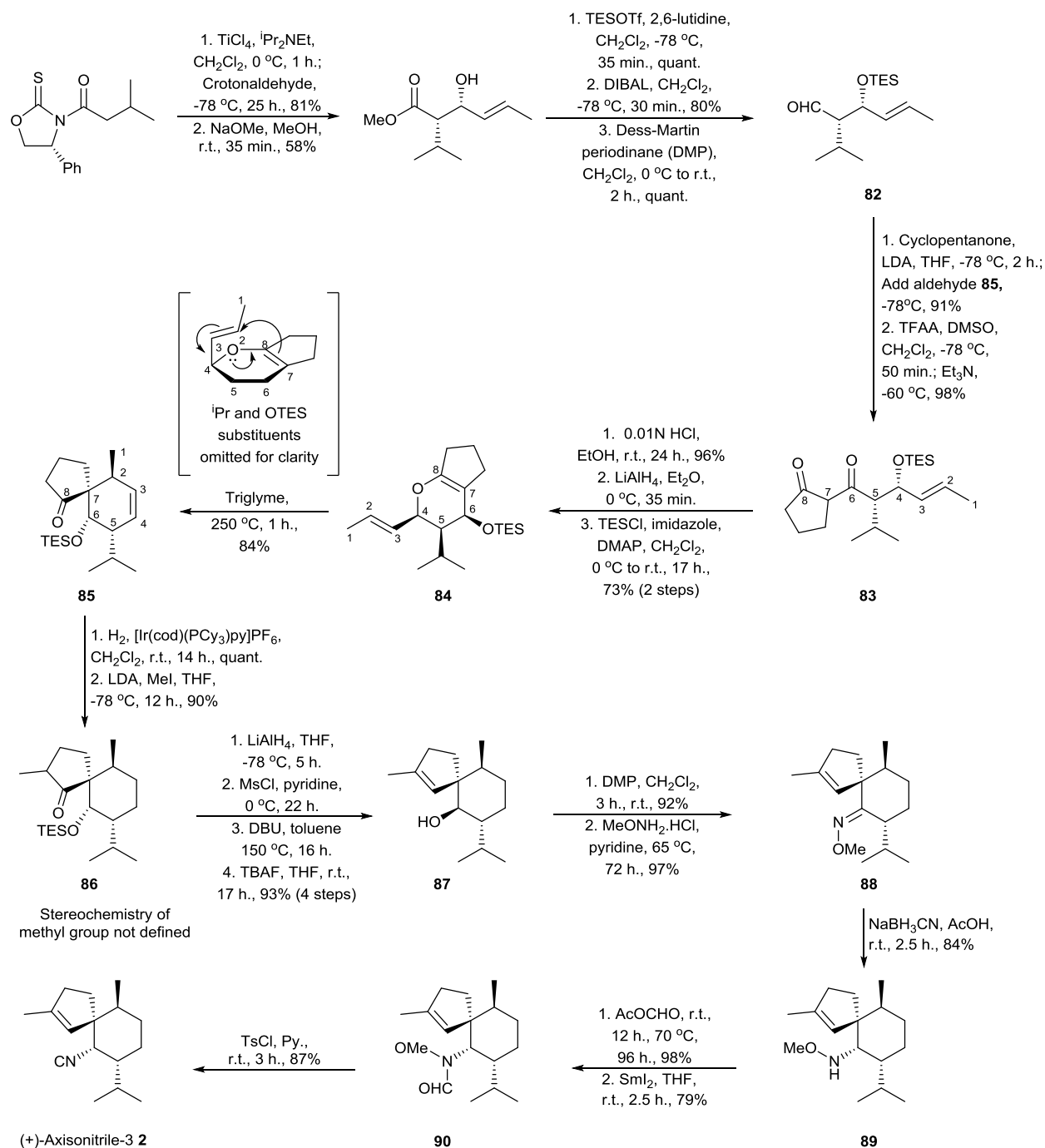
(+)-Axisonitrile-3 **2** has been isolated from the sponge *Axinella cannabina* and shown to demonstrate useful antimalarial activity,<sup>13,41</sup> that was subsequently shown to be due to coordination of its isocyanide fragment to iron in heme that helps avoid its sequestration into  $\beta$ -hematin, thus preventing the peroxidative and glutathione-mediated pathways that lead to heme destruction.<sup>42</sup> For the first synthesis of non-natural (-)-axisonitrile-3 **2** (Scheme 6),<sup>43</sup> Caine and Deutsch transformed bicyclic  $\beta$ -hydroxy-ketone **72** into dienol acetate **73** via treatment with acetic anhydride and a catalytic amount of sulphuric acid, which was then oxidised using mCPBA to afford hydroxy-enone **75** as a major diastereomer in 56% yield (as well as enone **74** in 42% yield). After chromatographic separation, the alcohol group of hydroxy-enone **75** was protected as its labile methoxyisopropylidene ether and a second alkene bond introduced via phenylselenenylation of its ketone enolate followed by selenoxide elimination/methoxymethyl ether (MOM) deprotection to afford dienone **76**. This dienone **76** was then irradiated as a dilute solution in dioxane which resulted in a remarkable photochemical skeletal rearrangement reaction (for mechanistic details see scheme 6)<sup>44</sup> to yield a single tricyclodecenone product **78**. The alkene bond of enone **78** was then hydrogenated, followed by a Wittig methylenation reaction to afford alkene **79**. Treatment of alkene **79** with lithium and ethylamine resulted in reductive ring-opening of the cyclopropane with inversion of configuration at C<sub>7</sub> to afford the spirocyclic ring system of a bicyclic alcohol that was converted into tosylate **80** via treatment with TsCl in pyridine. Tosylate **80** was treated with KN<sub>3</sub> in the presence of 18-crown-6, resulting in clean S<sub>N</sub>2 inversion to afford an azide intermediate that was reduced with LiAlH<sub>4</sub> to afford amine **81**. This amine **81** was then *N*-formylated with acetic formic anhydride to afford a formamide that was dehydrated with TsCl in pyridine to afford (-)-axisonitrile-3 **2**.<sup>43</sup>




 Scheme 6 Caine's synthesis of (-)-axisonitrile-3 **2**.<sup>43</sup>

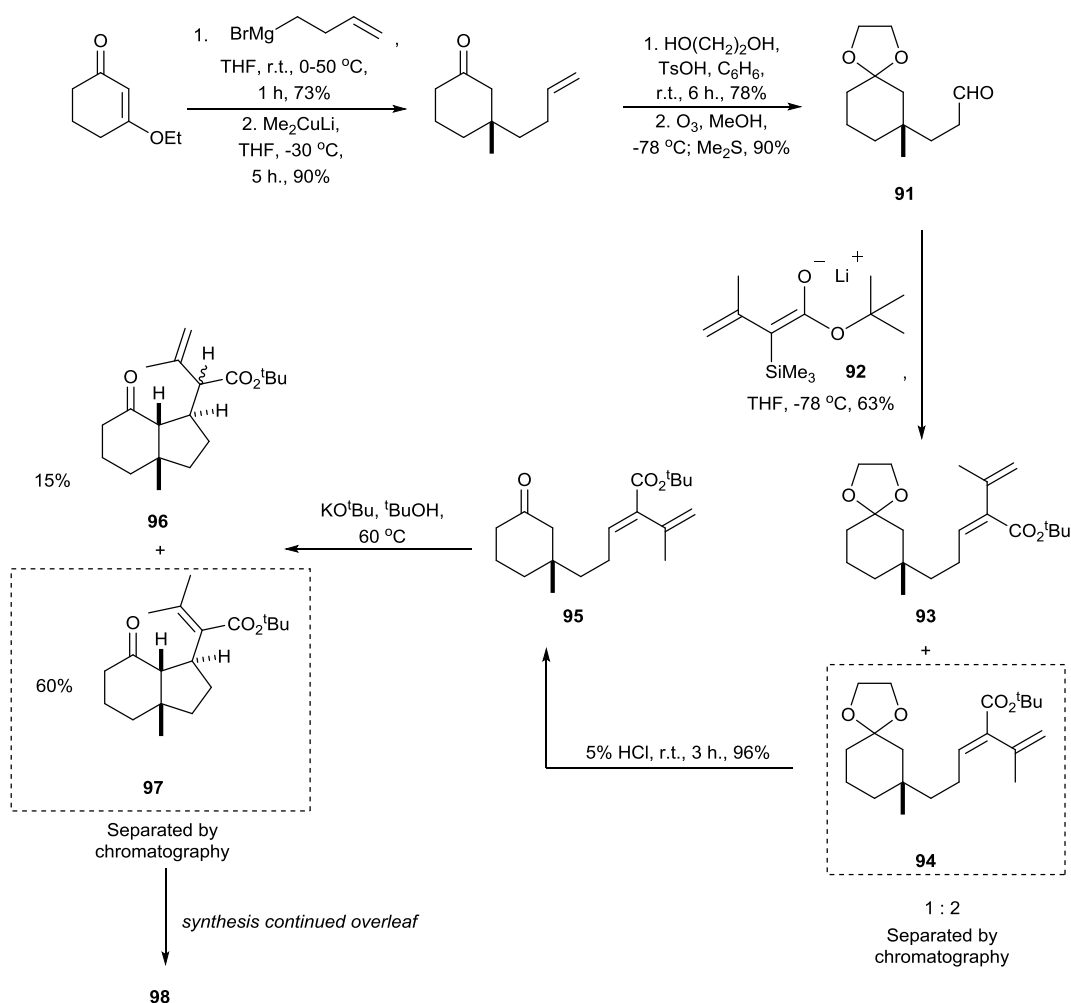
For their synthesis of natural (+)-axisonitrile **3 2** (Scheme 7), Kobayashi and co-workers used Evan's *syn*-aldol methodology to assemble chiral aldehyde **82**, which was reacted with the lithium enolate of cyclopentanone to afford an aldol product that was oxidised using trifluoroacetic anhydride (TFAA)/dimethyl sulfoxide (DMSO) conditions to give 1,3-diketone intermediate **83**. Treatment of this diketone **83** with mild acid resulted in a C<sub>7</sub>- enol intermediate that underwent an intramolecular *O*-alkylation/allylic substitution reaction to afford a cyclic enol ether whose keto group was reduced to afford an alcohol that was *O*-silyl protected to afford bicyclic enol-ether **84**. The key spiro[4,5]decane skeleton of ketone **85** was then accessed in a stereoselective manner *via* thermal Claisen rearrangement of unsaturated bicyclic tetrahydropyran **84**. Ketone **85** was then converted into oxime **88** in eight straightforward steps involving: (i) alkene hydrogenation; (ii) enolate methylation to afford ketone; (iii) ketone reduction; (iv) and (v) E<sub>2</sub>-elimination of a mesylate; (vi) *O*-silyl deprotection

to afford alcohol; (vii) alcohol oxidation; (viii) formation of (*E*)-*O*-methyl-oxime **86**. Stereoselective reduction of *O*-methyl oxime **86** with NaBH<sub>3</sub>CN afforded methoxyamine **89** with good levels of stereocontrol, which was then transformed into an *N*-methoxy-formamide **90** via treatment with acetic formic anhydride. Samarium iodide was then used for reductive *N*-*O* bond cleavage to afford an *N*-*H*-formamide intermediate that was dehydrated with TsCl in pyridine to afford (+)-axisonitrile-3 **2**.<sup>45</sup>

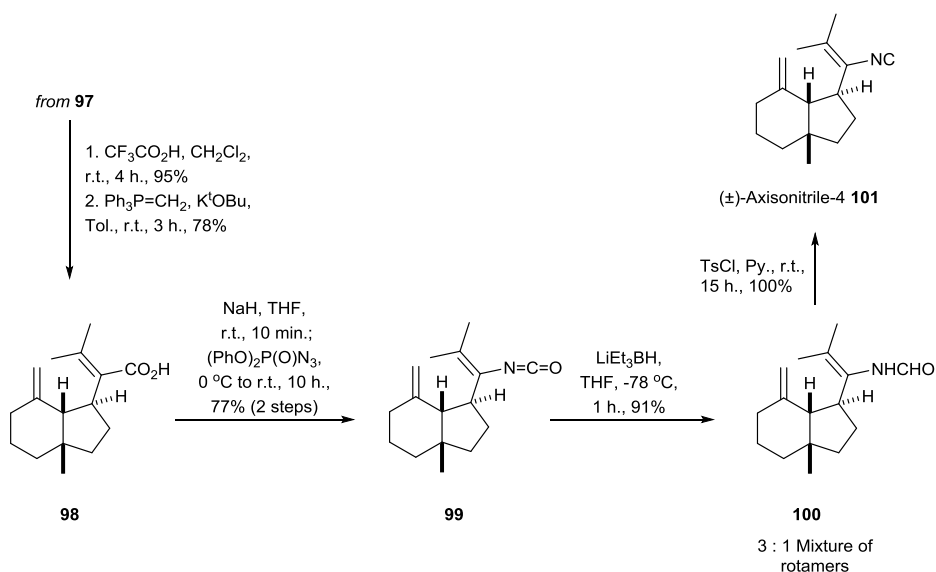


Scheme 7 Kobayashi's synthesis of (+)-axisonitrile-3 **2**.<sup>45</sup>

Hart and co-workers have also published a synthesis of (±)-axisonitrile-4 **101** (Scheme 8) employing a similar strategy to that reported for their synthesis of (±)-axisonitrile-1 **55** (see Scheme 4).<sup>46</sup> Peterson olefination of the lithium enolate of *tert*-butyl 3-methyl-2-(trimethylsilyl)-3-butenolate **92** with aldehyde **91** resulted in a 1 : 2 mixture of diene esters **93** and **94** that could be separated by chromatography. Acid catalysed acetal deprotection of acetal **94** afforded ketone **95** that underwent base-catalysed intramolecular conjugate addition reaction to afford the desired tetrasubstituted alkene **97** and the unwanted disubstituted alkenes **96** (as a mixture of epimers). Tetrasubstituted alkene **97** was purified by chromatography and then hydrolysed with TFA, followed by Wittig methylenation to afford acid **98**. This acid **98** was then transformed into its corresponding formamide **100** using a reductive Curtius rearrangement protocol involving reaction of its carboxylate with diphenyl phosphorazidate (DPPA), followed by reduction of the resultant isocyanate **99** with LiEt<sub>3</sub>BH (Super-Hydride). Formamide **100** was then dehydrated into its corresponding isocyanide *via* treatment with TsCl and pyridine to afford (±)-axisonitrile-4 **101**.<sup>46</sup>

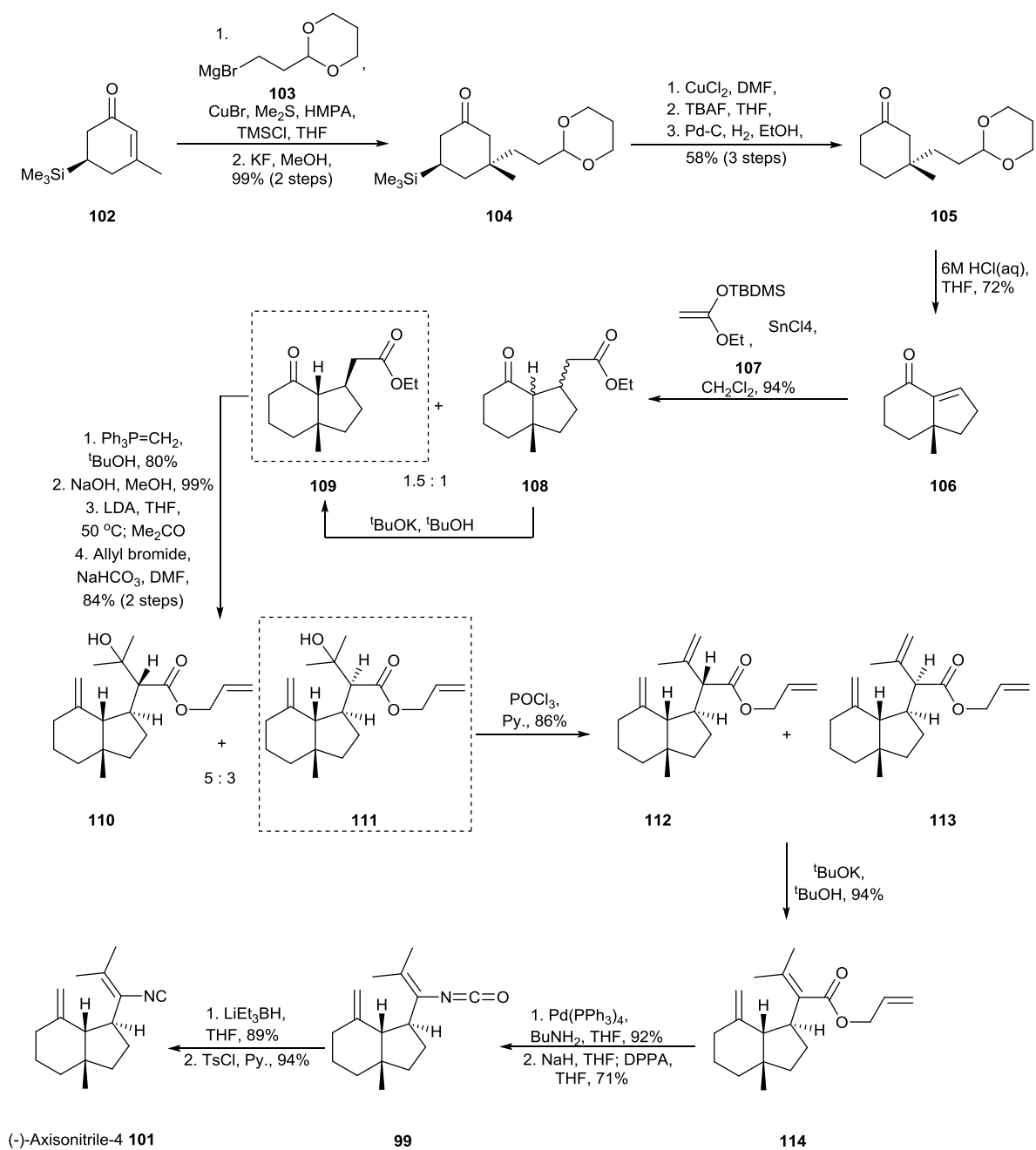




Scheme 8 Hart's synthesis of (±)-axisonitrile-4 **101**.<sup>46</sup>

Asaoka and co-workers reported an enantioselective synthesis of (-)-axisonitrile-4 **101**<sup>47</sup> starting with stereoselective conjugate addition of the cuprate of Grignard reagent **104** to enone **102** to afford the quaternary stereocentre of ketone **104** (Scheme 9). The silyl fragment of enone **104** was then eliminated *via* sequential treatment with  $\text{CuCl}_2$  and TBAF to afford an enone intermediate that was then hydrogenated to afford ketone **105**. Treatment of ketone **105** with 6M  $\text{HCl}_{(\text{aq})}$  resulted in acetal deprotection to afford an aldehyde intermediate that underwent a spontaneous intramolecular aldol condensation reaction to afford bicyclic enone **106**. This enone **106** then underwent  $\text{SnCl}_4$  catalysed intermolecular conjugate addition reaction with silyl ketene acetal **107** to generate a 1 : 1.5 mixture of inseparable diastereomeric esters **108** and **109**. However, the esters **111** could be selectively epimerised into their thermodynamically more stable isomer **109** *via* simple treatment with potassium *tert*-butoxide. The resultant ester **109** was then reacted with  $\text{Ph}_3\text{PC}=\text{CH}_2$  to afford an alkene whose ester group was hydrolysed under basic conditions to afford an acid intermediate. The dianion of this acid intermediate then underwent an aldol reaction with acetone, followed by base catalysed esterification with allyl bromide to generate a 5 : 3 mixture of tertiary alcohols **110** and **111**, which were dehydrated with  $\text{POCl}_3$  to afford the terminal alkenes **112** and **113**. Treatment of the mixture of alkenes **112** and **113** with potassium *tert*-butoxide resulted in alkene isomerisation to afford the thermodynamically more stable tetrasubstituted alkene **114**, which was transformed into isocyanate **99** *via* palladium catalysed allyl deprotection, followed by Curtius rearrangement of a derived acyl azide intermediate. The resultant isocyanate **99** was then reduced with  $\text{LiEt}_3\text{BH}$  to afford a formamide intermediate that was dehydrated with TsCl in pyridine to generate (-)-axisonitrile-4 **101**.<sup>47</sup>

Methodology for the synthesis of isocyanide containing natural products

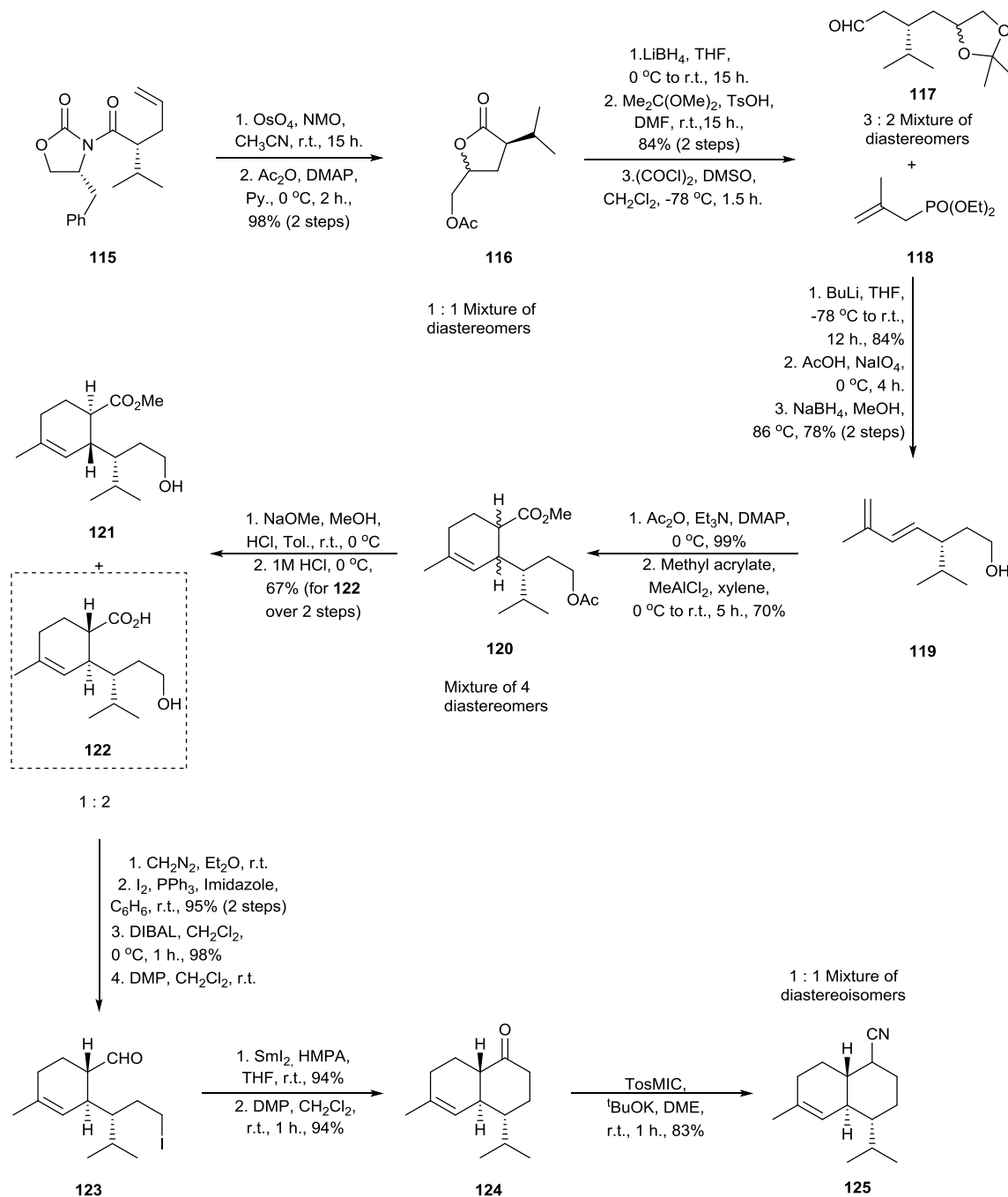


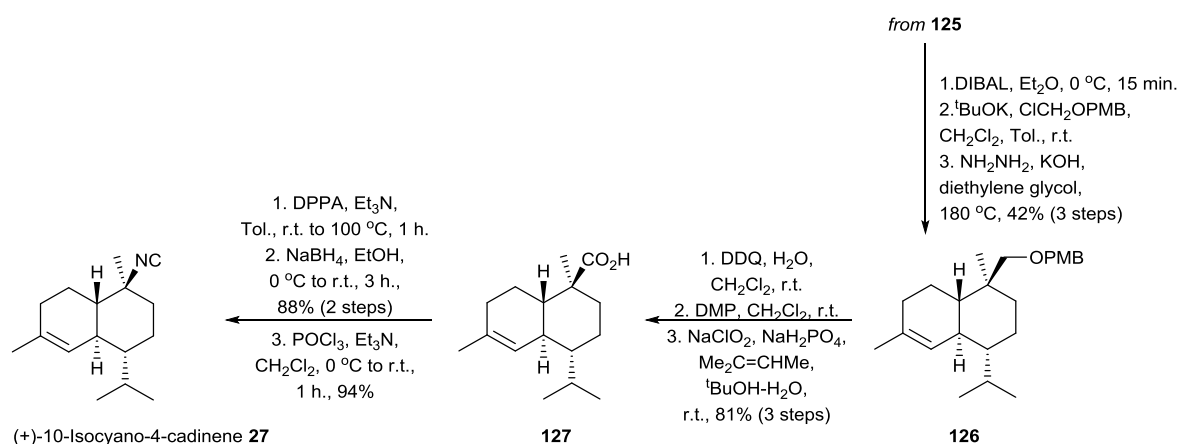
Scheme 9 Asaoka's synthesis of (-)-axisonitrile-4 **101**.<sup>47</sup>

**1.1.3. Syntheses of isocyano-4-cadinene, isocyanoisodauc-5-ene, isocyanopupukeanane and isocyanotrachyospane derived isocyanide natural products**

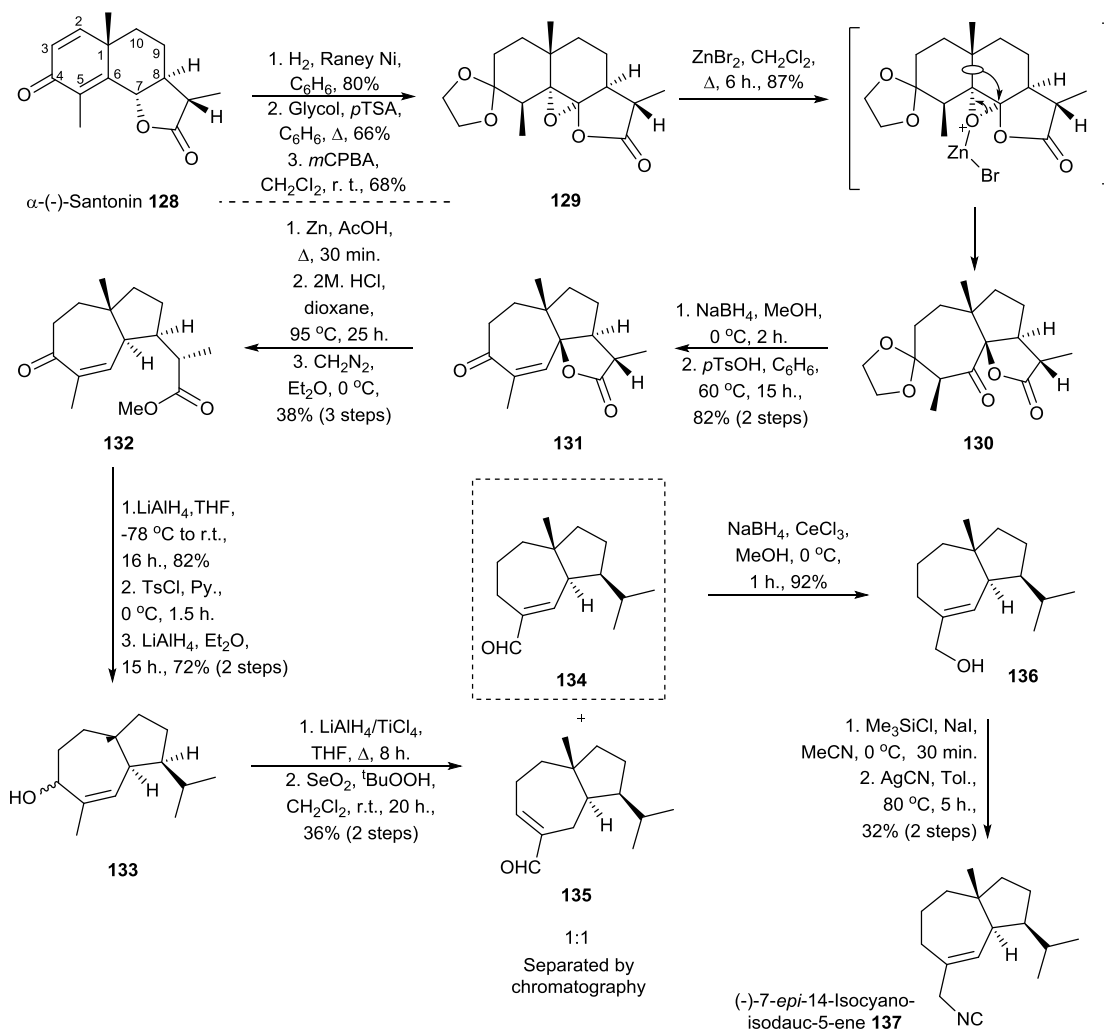
Matsuda and co-workers described the first enantioselective synthesis of (+)-10-isocyano-4-cadinene **27** (Scheme 10),<sup>28,47</sup> a marine sesquiterpene with antifouling activities against barnacles, which was isolated from the nudibranch *Phyllidiidae*.<sup>48</sup> Dihydroxylation of the alkene functionality of Evans' *N*-acyl-oxazolidin-2-one **115** resulted in spontaneous lactonisation of the resultant mixture of epimeric diols to afford a 1 : 1 mixture of 5-membered lactones whose primary alcohol groups were acetylated with acetic anhydride to afford a mixture of epimeric acetates **116**. This mixture of acetates **116** was converted into a mixture of epimeric triols *via* global reduction using LiBH<sub>4</sub>, followed by selective acetonide protection of their 1,2-diol fragments, followed by Swern oxidation of their remaining primary alcohol groups to afford a 3 : 2 mixture of epimeric aldehydes **117**. Horner–Wadsworth–Emmons reaction of this mixture of epimeric aldehydes **117** with the lithium anion of diethyl 2-methyl-2-propenyl phosphonate **118** afforded a diene intermediate that was subjected to a one-pot deprotection of its acetonide group. Oxidative cleavage of the resultant diol with NaIO<sub>4</sub> afforded an aldehyde intermediate that was reduced with NaBH<sub>4</sub> into alcohol **119**. This alcohol **119** was protected as its acetate, which was then reacted with methyl acrylate in the presence of MeAlCl<sub>2</sub>, resulting in an intermolecular Diels-Alder reaction to afford cyclohexenyl acetate **120** as a mixture of four diastereomers. This mixture of diastereomeric acetates was equilibrated *via* treatment with NaOMe in methanol to afford an inseparable mixture of diastereomeric *trans*-esters, which could be separated *via* treatment with 1M HCl at 0 °C resulting in selective hydrolysis of the desired *trans*-ester to afford its corresponding *trans*-acid. After separation from the unreacted *trans*-ester **121**, *trans*-acid **122** was then esterified *via* treatment with diazomethane, its primary alcohol group converted into an iodide, and its ester group reduced with diisobutylaluminium hydride (DIBAL) to afford an alcohol that was then oxidised to its corresponding aldehyde **123** *via* treatment with Dess-Martin Periodinane (DMP). A samarium (II) iodide mediated Barbier cyclisation reaction was then employed to generate a primary radical species from the iodide functionality of **123** that underwent intramolecular cyclisation onto its aldehyde group to afford a cyclohexanol intermediate that was oxidised to its corresponding ketone **124** using DMP. Ketone **124** was then treated with the anion of *para*-toluenesulfonylmethyl isocyanide (TOSMIC), which resulted in formation of a 1 : 1 mixture of epimeric cyanides **125**. Reduction of this mixture of cyanides **125** with DIBAL afforded a mixture of their corresponding aldehydes, whose potassium enolate was reacted with *para*-methoxybenzyl chloromethyl ether to afford an aldehyde intermediate containing a

quaternary  $\alpha$ -stereocentre. The resultant aldehyde was reduced under Wolff-Kishner conditions to afford *para*-methoxybenzyl (PMB) ether **126**, whose PMB ether group was cleaved oxidatively using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), followed by sequential Dess-Martin and Pinnick oxidation to afford acid **127**. This acid **127** was then converted into (+)-10-isocyano-4-cadinene **27** via a three step protocol involving (i) Curtius rearrangement of an acyl azide intermediate; (ii)  $\text{NaBH}_4$  reduction of the resultant isocyanate; (iii)  $\text{POCl}_3/\text{Et}_3\text{N}$  mediated dehydration of the resultant formamide.<sup>47</sup>



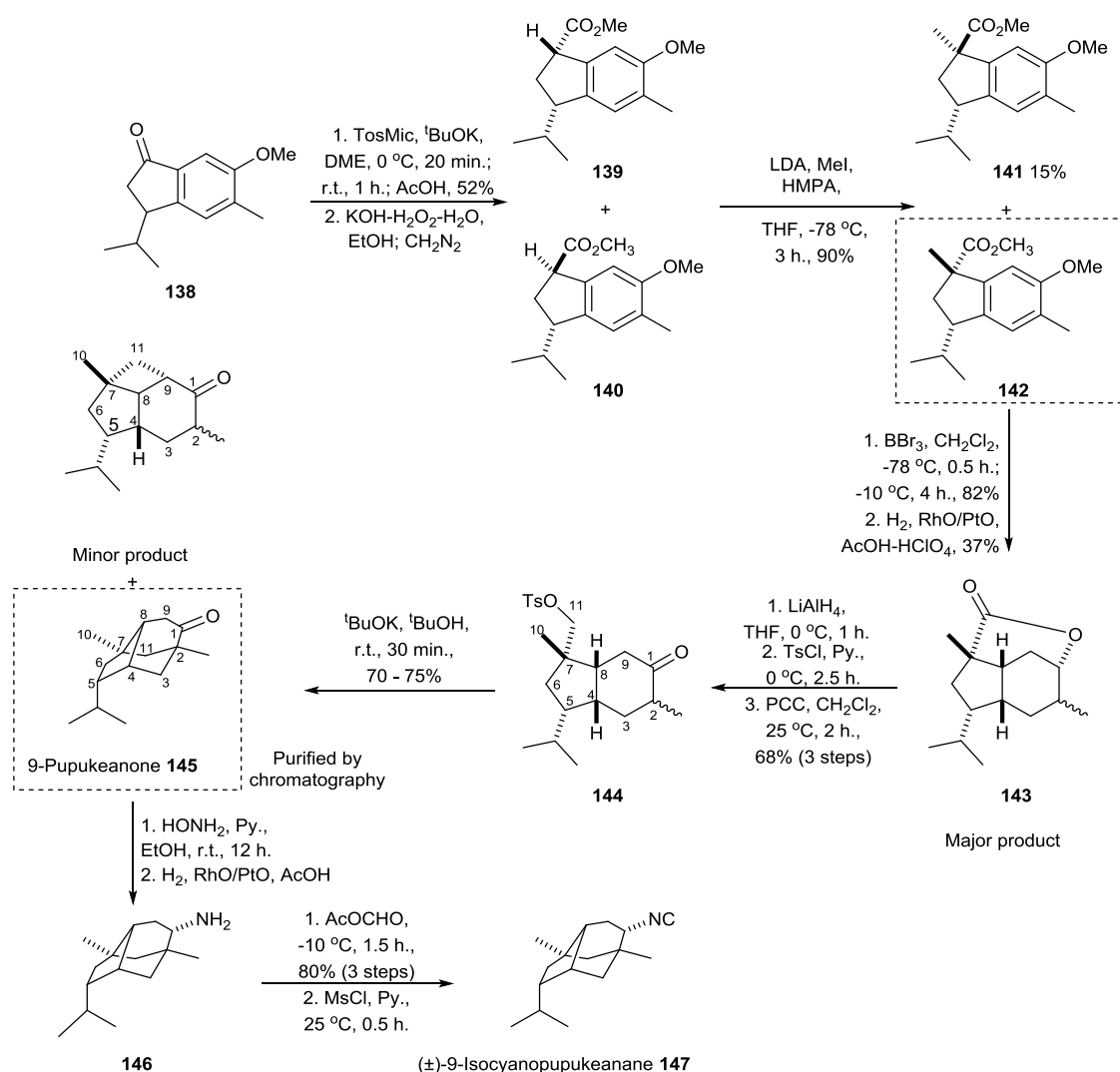

 Scheme 10 Matsuda's Synthesis of (+)-10-isocyano-4-cadinene **27**.<sup>28,48</sup>

In 2003, Tu and co-workers utilised silver cyanide as a nucleophile to introduce the isocyanide functionality of the guaiane sesquiterpene (-)-7-*epi*-14-isocyano-isodauc-5-ene **137** (Scheme 11),<sup>50</sup> which was first isolated from the methanol extract of the marine sponge *Acanthella acuta*.<sup>51</sup>  $\alpha$ -(-)-Santonin **128** was converted into epoxide **129** via a three step protocol involving selective 2,3-alkene hydrogenation, acetal protection/alkene bond migration [(5,6)-alkene  $\rightarrow$  (6,7)-alkene] and stereoselective epoxidation.<sup>52</sup> This epoxide **129** then underwent a ZnBr<sub>2</sub> mediated epoxide ring opening/ring expansion reaction to afford the new [5.3.0] ring system of lactone **130**. Ketone **130** was then reduced with NaBH<sub>4</sub>, followed by acid catalysed ketal deprotection and dehydration of the resultant hydroxyl group to afford the alkene bond of enone **131**. Reductive cleavage of the lactone ring of enone **131** using Zn/AcOH was followed by acid catalysed alkene isomerisation to afford an enone whose acid group was esterified using diazomethane to afford ester **132**. This ester **132** was reduced to give a diol whose primary alcohol functionality was tosylated and reduced with LiAlH<sub>4</sub> to give the isopropyl fragment of alcohol **133**. Subsequent reductive deoxygenation (LiAlH<sub>4</sub>/TiCl<sub>4</sub>) of the allylic alcohol functionality of **133** resulted in formation of a pair of regioisomeric alkenes whose allylic methyl groups were oxidised with SeO<sub>2</sub> to afford a 1 : 1 mixture of enals **134** and **135**. Regioisomer **134** was then purified by chromatography and reduced to afford allylic alcohol **136**, whose alcohol functionality was converted into its corresponding allylic iodide via treatment with NaI in the presence of trimethylsilyl chloride (TMSCl). Finally, treatment of this iodide intermediate with silver cyanide resulted in nucleophilic displacement to afford the isocyanide functionality of (-)-7-*epi*-14-isocyano-isodauc-5-ene **137**.<sup>50</sup>


 Scheme 11 Tu's synthesis of (-)-7-epi-14-isocyano-isodauc-5-ene **137**.<sup>50</sup>

(-)-9-Isocyanopupukeanane **147** is an allomone produced by the sponge *Hymeniacidon* sp. that accumulates in the nudibranch *Phyllidia varicosa*, which was first isolated *via* extraction of the foul smelling mucus of these molluscs.<sup>53</sup> It is believed to be used by both species as a defence secretion, with its synthesis in racemic form having first been reported by Corey *et al.* in 1979 (Scheme 12).<sup>54</sup> In this synthesis, indan-1-one **138** was reacted with TosMIC to afford a mixture of diastereomeric  $\text{C}_1$ -homologated nitrile intermediates that were hydrolysed into their parent acids followed by esterification to afford their diastereomeric methyl esters **139/140**, *via* sequential treatment with potassium hydroperoxide/hydroxide and  $\text{CH}_2\text{N}_2$ . Deprotonation of this mixture of esters **139/140** afforded an enolate that was alkylated with  $\text{MeI}$  to give  $\alpha$ -methyl ester **142** (and 15% of its (*cis*)-epimer **141**), which upon treatment with boron tribromide resulted in cleavage of both its methyl ester and methyl ether groups to give a phenolic acid. This acid was then hydrogenated with Nishimura's catalyst ( $\text{RhO}/\text{PtO}$ ) in the presence of  $\text{AcOH}-\text{HClO}_4$  to afford lactone **143** as the major product. Lactone **143** was then

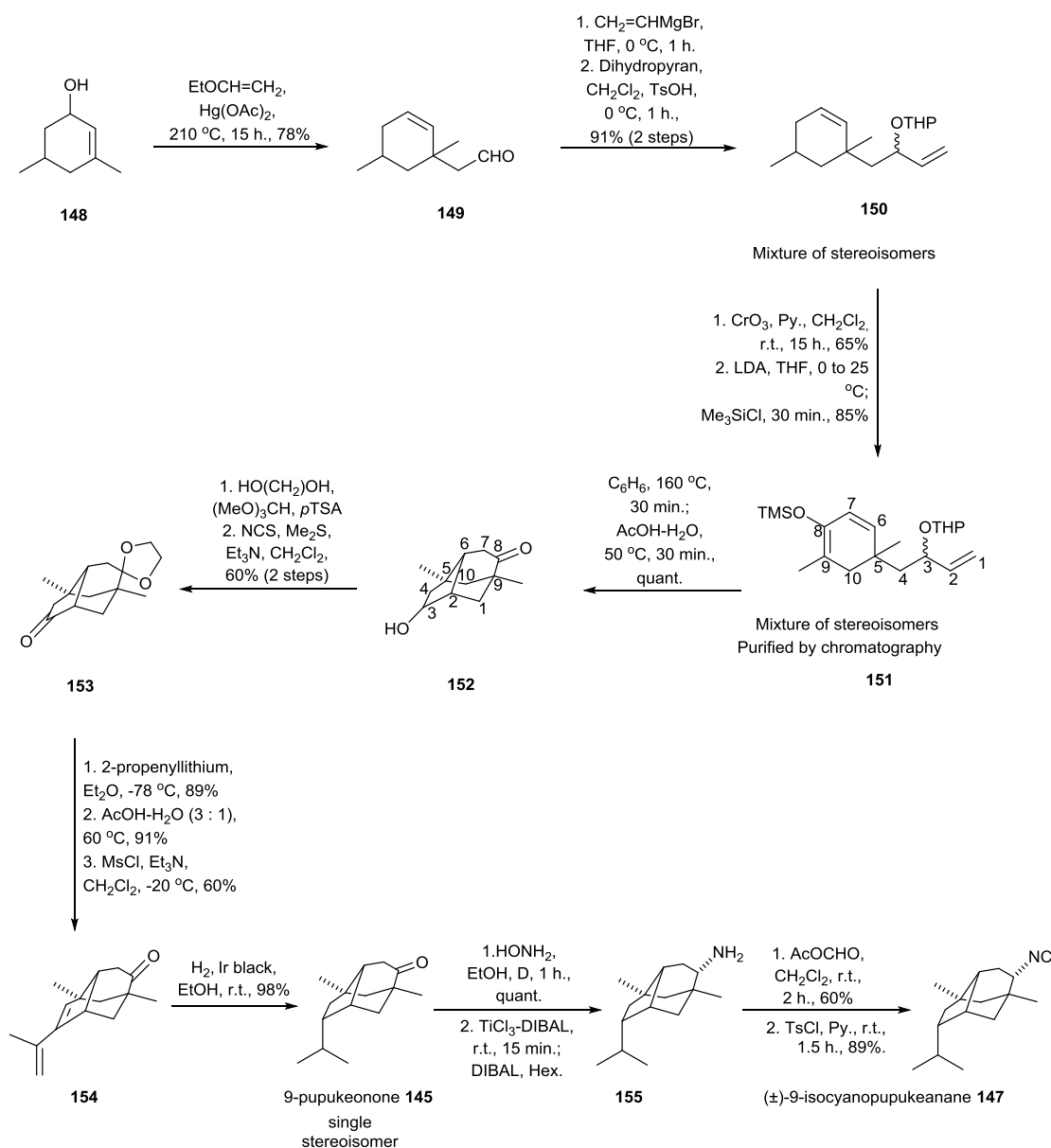
reduced with  $\text{LiAlH}_4$  to afford a diol whose primary alcohol functionality was mono-tosylated, and whose secondary alcohol was oxidised with pyridinium chlorochromate (PCC) to generate the desired ketone **144**. Treatment of ketone **144** with potassium *tert*-butoxide afforded an enolate that underwent intramolecular nucleophilic displacement of its tosyl group to give the spirocyclic core of ketone **145** as the major product. After separation, ketone **145** was then converted into an oxime *via* treatment with hydroxylamine, which was hydrogenated with Nishimura's catalyst to afford amine **146**. This amine **146** was then *N*-formylated with acetic formic anhydride to generate a formamide that was dehydrated *via* treatment with methanesulfonylchloride  $\text{MsCl}$  in pyridine to give the natural product ( $\pm$ )-9-isocyanopupukeanane **147**.<sup>54</sup>



Scheme 12 Corey's synthesis of ( $\pm$ )-9-isocyanopupukeanane **147**.<sup>54</sup>

A second synthesis of (±)-9-isocyanopupukeanane **147** was reported simultaneously by Yamamoto and Sham in 1979, with the construction of the core ring being achieved *via* an intramolecular Diels-Alder reaction of triene **151** (Scheme 13).<sup>55</sup> Therefore, Claisen rearrangement of a vinyl-ether, prepared *in situ* by treatment of alcohol **148** with mercuric acetate and vinyl ethoxid, afforded an aldehyde **149** which was reacted with vinyl magnesium bromide, followed by tetrahydropyran (THP) protection, to afford *O*-THP protected diene **150** as a mixture of stereoisomers. Treatment of this mixture of dienes **150** with CrO<sub>3</sub> resulted in allylic oxidation to afford a mixture of enone intermediates that were protected as their *O*-silyl enol ethers *via* treatment with LDA/TMSCl, which after purification by chromatography gave triene **151** as a single isomer. An intramolecular Diels-Alder cyclisation reaction was then initiated *via* heating triene **151** in benzene in the presence of acetic acid to afford the highly strained ring system of ketone **152**. Ketone **152** was then reacted with ethylene glycol to afford a cyclic ketal, whose secondary alcohol functionality was oxidised (NCS, Me<sub>2</sub>S, Et<sub>3</sub>N) to afford ketone **153**. Ketone **153** was then reacted with 2-propenyllithium to afford an alcohol whose ketal group was deprotected and its tertiary alcohol group mesylated/eliminated to afford the diene functionality of ketone **154**. Ketone **154** was then globally hydrogenated using an iridium black catalyst to afford isopropyl ketone **145** as a single diastereomer, whose corresponding oxime was then reduced using a mixture of TiCl<sub>3</sub>/DIBAL to afford an imine that was further reduced with DIBAL to afford amine **155** as a single diastereomer. Formylation of amine **155** using acetic formic anhydride, followed by dehydration of the resulting formamide using TsCl and pyridine then gave the isocyanide natural product (±)-9-isocyanopupukeanane **145**.<sup>55</sup> Both Corey's and Yamamoto's syntheses of (±)-9-isocyanopupukeanane **147** (schemes 12 and 13) proceed through 9-pupukeanone **145** as a late stage intermediate, which has since been targeted by a number of groups as a synthon for (±)-9-isocyanopupukeanane **147**'s synthesis.<sup>56–59</sup>

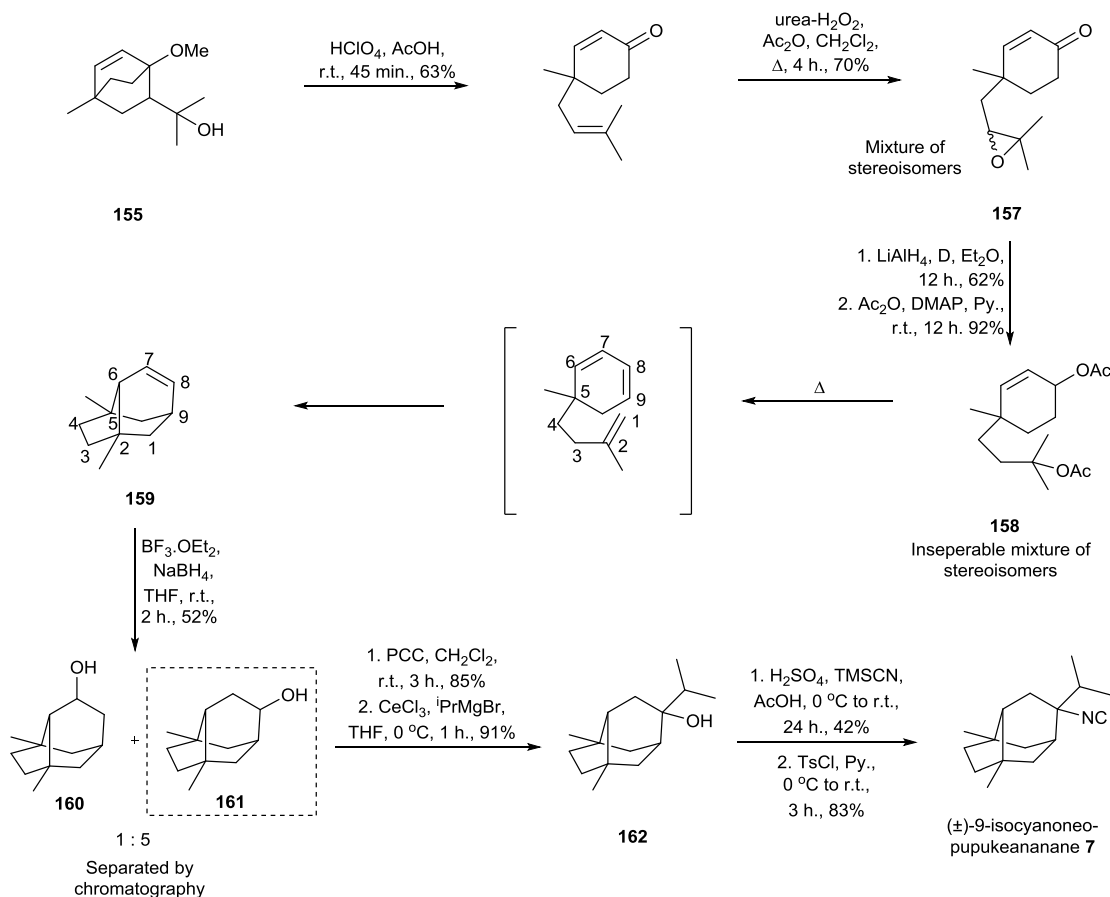




Scheme 13 Yamamoto's synthesis of (±)-9-isocyanopupukeanane **147**.<sup>55</sup>

Ho and Jana have reported the synthesis of the structurally related (±)-9-isocyanoneopupukeanane **7** using bicyclic tertiary alcohol **156** as starting material (Scheme 14), which was treated with a mixture of perchloric and acetic acid resulting in an acid-catalysed fragmentation reaction to afford an enone, whose alkene side-chain was epoxidised using hydrogen peroxide-urea in acetic anhydride to afford a mixture of epoxide diastereomers **157**. This mixture of epoxides **157** was globally reduced with LiAlH<sub>4</sub> to afford a mixture of diols that were *bis*-acetylated to afford a mixture of diacetates **158**. Upon heating in a sealed tube, this mixture of diacetates **158** underwent a *bis*-elimination reaction to afford a triene intermediate *in situ*, which cyclised *via* an intramolecular Diels-Alder reaction to afford tricyclic olefin **159** that was then hydroborated/oxidised to afford a 1 : 5 mixture of

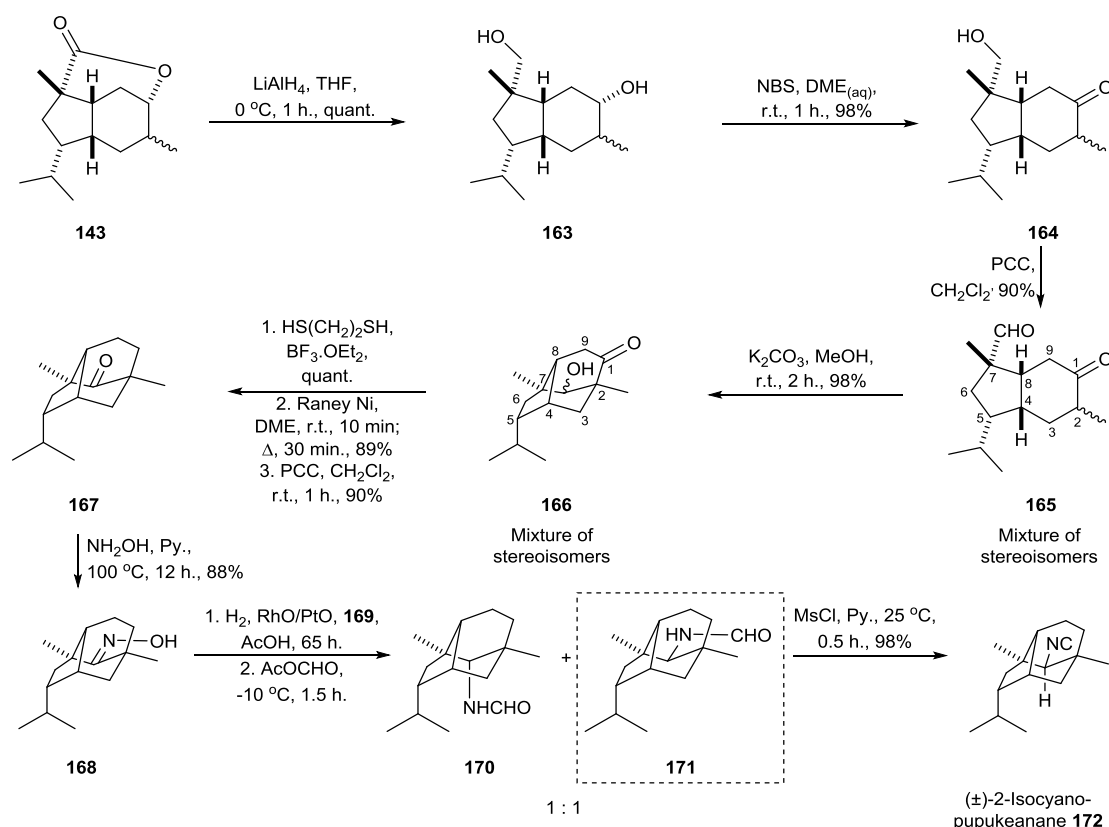
regioisomeric alcohols **160** and **161**. Alcohol **161** was purified by chromatography and then oxidised with pyridinium chlorochromate (PCC) to produce a ketone intermediate that was reacted with with  $i\text{PrMgBr}$  to afford tertiary alcohol **162**. Alcohol **162** then underwent a Ritter reaction with  $\text{TMSCN}$  in the presence of sulfuric acid to afford a formamide intermediate that was dehydrated using  $\text{TsCl}$  in pyridine to afford ( $\pm$ )-9-isocyanoneopupukeanane **7**.<sup>60</sup>



Scheme 14 Ho's synthesis of ( $\pm$ )-9-isocyanoneopupukeanane **7**.<sup>60</sup>

Corey utilised his previously synthesised lactone **143** as an advanced starting material for the synthesis of ( $\pm$ )-2-isocyanopupukeanane **171** (Scheme 15)<sup>61</sup>, reducing it to its corresponding diol **163** using  $\text{LiAlH}_4$ , followed by selective oxidation of its secondary alcohol functionality using NBS to generate  $\delta$ -hydroxy ketone **164**. This ketone **164** was oxidised with PCC to give aldehyde **165**, which underwent a base catalysed intramolecular aldol reaction to afford tricyclic aldol **166** as the major product. Treatment of ketone **166** with ethanedithiol and catalytic  $\text{BF}_3 \cdot \text{OEt}_2$  gave a thioketal intermediate that underwent desulphurisation on exposure to Raney nickel in ethanol to afford an alcohol intermediate that was oxidised to its

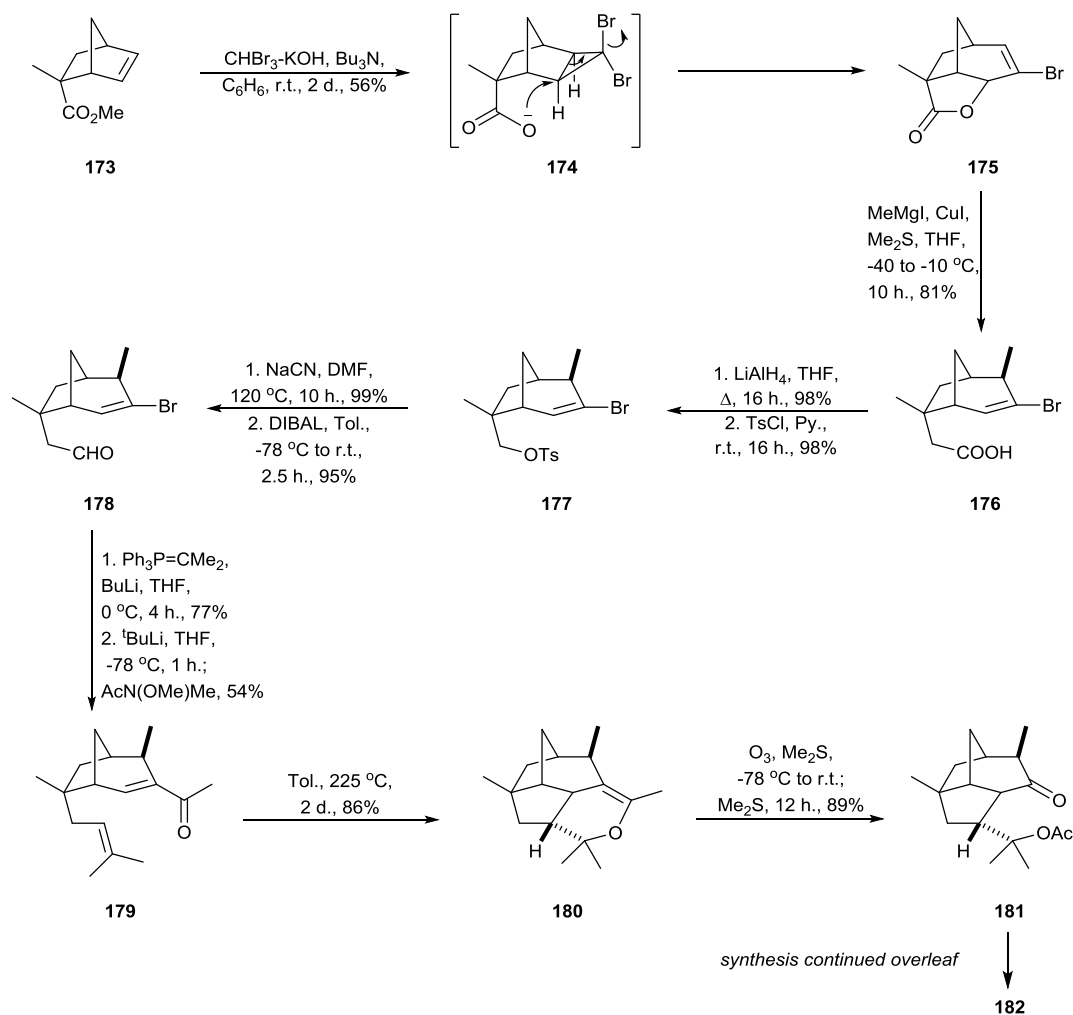
corresponding ketone **167** using PCC. This ketone **167** then underwent reaction with excess hydroxylamine to afford an oxime **168** that was reduced with Nishimura's catalyst **169** to afford a 1 : 1 mixture of epimeric amines that were converted into their corresponding formamides **170** and **171** via treatment with formyl acetic acid. This mixture of formamides was separated by chromatography, and formamide **171** then converted into (±)-2-isocyanopupukeanane **172** via treatment with MsCl in pyridine.<sup>61</sup> A variety of synthetic strategies have since emerged for the synthesis of 2-pupukeanone **167**, which was an advanced intermediate in Corey's synthesis of 2-isocyanopupukeanane **172**.<sup>62–72</sup>

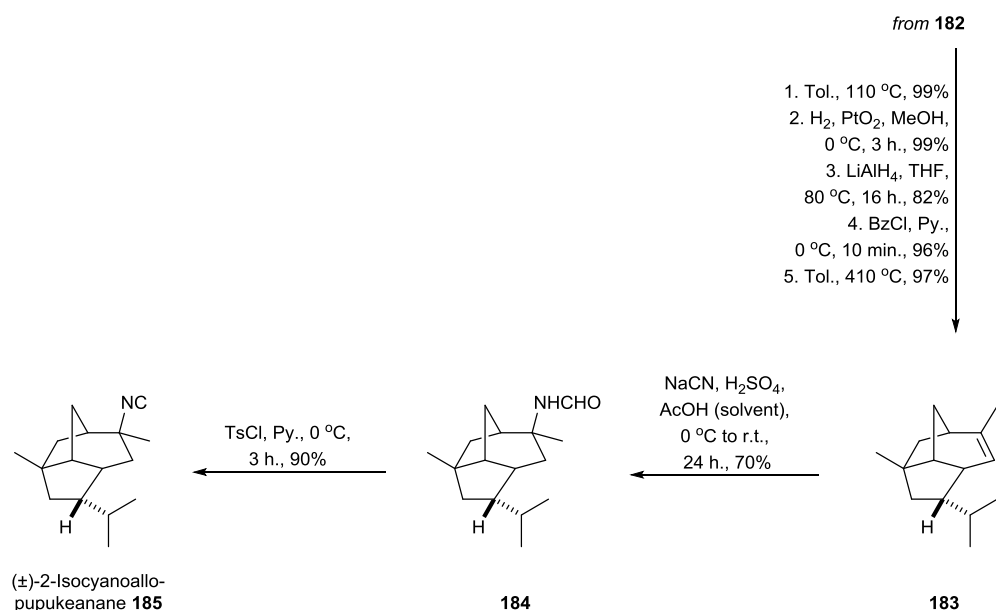


Scheme 15 Corey's synthesis of (±)-2-isocyanopupukeanane **172**.<sup>61</sup>

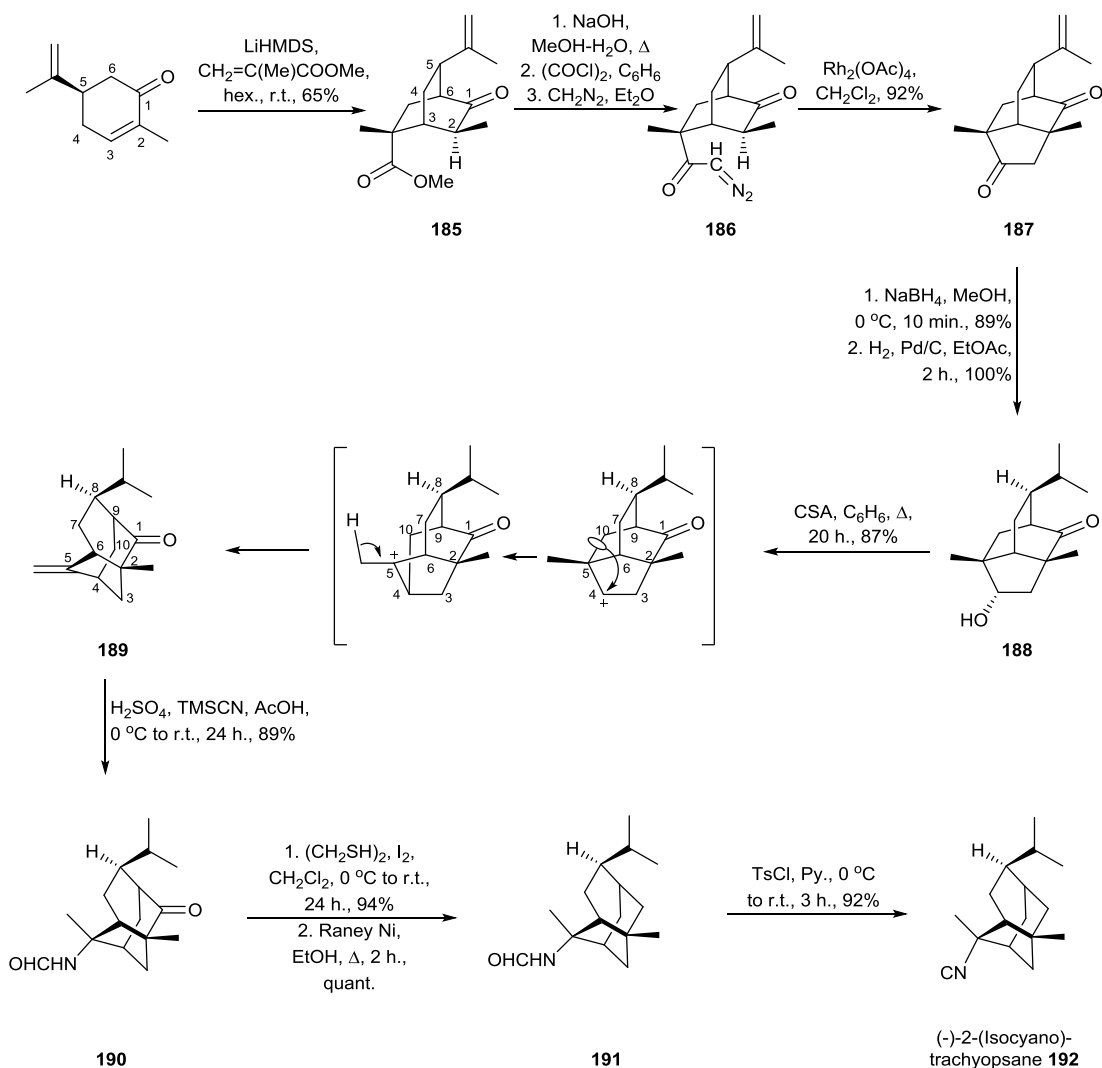
Ho and co-workers have also reported the synthesis of 2-isocyanopallopupukeanane **184**, a marine sponge sesquiterpene from *Cioca-lypta* sp.,<sup>53</sup> which is closely related to *Hymeniacidon* sp. (Scheme 16).<sup>60,73</sup> Base catalysed hydrolysis of ester **173** afforded a carboxylate intermediate that underwent a phase transfer catalysed dibromocarbene mediated cyclopropanation reaction to afford a dibromocyclopropane intermediate **174** that underwent a lactonisation/cyclopropyl ring-opening/bromide elimination reaction to afford tricyclic lactone **175**. This lactone **175** was treated with MeMgI and copper iodide, resulting in an S<sub>N</sub>2 substitution reaction to afford acid **176**. Reduction of acid **176** with LiAlH<sub>4</sub>, followed by

treatment of the resultant alcohol with TsCl/pyridine afforded tosylate **177**, which upon reaction with NaCN gave a nitrile that was reduced with DIBAL to give aldehyde **178**. Wittig methylenation of aldehyde **178** afforded a vinyl bromide intermediate that was metalated with *tert*-butyl lithium followed by treatment with AcN(OMe)Me to generate diene ketone **179**. This ketone **179** was heated in toluene in a sealed tube at 225 °C to facilitate an intramolecular hetero-Diels-Alder reaction to afford tetracyclic enol ether **180**. Ozonolysis of enol ether **180** resulted in ring cleavage to generate ketone **181**, which was converted into formamide **183** over steps including: (i) thermal E<sub>2</sub>-elimination of its acetate group; (ii) hydrogenation of the resultant alkene bond; (iii) LiAlH<sub>4</sub> reduction of its keto group; (iv) *O*-benzoylation of the resultant secondary alcohol group; (iv) thermal E<sub>2</sub>-elimination of the *O*-benzoate group. The alkene functionality of ketone **182** then underwent a Ritter reaction with NaCN in the presence of acetic acid to afford formamide **183**, which was subsequently dehydrated using TsCl and pyridine to afford the natural product (±)-2-isocyanoallopupukeanane **185**.<sup>60,73</sup>



Scheme 16 Ho's synthesis of (±)-2-isocyanoallopupukeanane **185**.<sup>60,73</sup>

Srikrishna *et al.* have also reported the synthesis of (-)-2-(isocyano)trachyopsane **192**,<sup>74</sup> whose antipode was isolated from the nudibrach *Phyllidi varicosa* and shown to have reasonable antifouling activity.<sup>48</sup> For its total synthesis (Scheme 17),<sup>74</sup> the lithium enolate of (*R*)-(-)-carvone underwent a stereoselective intermolecular conjugate addition reaction to methyl methacrylate, with the resultant enolate intermediate ring closing on itself at its C<sub>3</sub>- position to afford the bicyclo[2.2.2]octanecarboxylate skeleton of ester **185**. This ester **185** was then transformed into its corresponding diazoketone **186** (COCl<sub>2</sub>, CH<sub>2</sub>N<sub>2</sub>) that was used as a precursor to generate a highly reactive rhodium carbenoid species that underwent a regioselective intramolecular insertion reaction at its C<sub>2</sub>- axial C-H bond to afford the tricyclic ring skeleton of diketone **187**. Diketone **187** was then regio- and stereoselectively reduced using NaBH<sub>4</sub> to afford an alcohol intermediate whose alkene functionality was hydrogenated to afford the isopropyl fragment of alcohol **188**. Heating alcohol **188** with camphor sulphonic acid (CSA) in benzene resulted in a highly regioselective biomimetic acid catalysed rearrangement of its neopukeane skeleton to afford the trachyopsane skeleton of **189**. The formamide functionality of **190** was then introduced *via* an acid catalysed Ritter reaction, with its keto group then being reduced out in two steps *via* formation of a thioketal that underwent desulfurisation with Raney nickel to afford formamide **191**. Subsequent dehydration of formamide **191** with TsCl in pyridine resulted in formation of *ent*-2-(isocyano)trachyopsane **192**.<sup>74</sup>

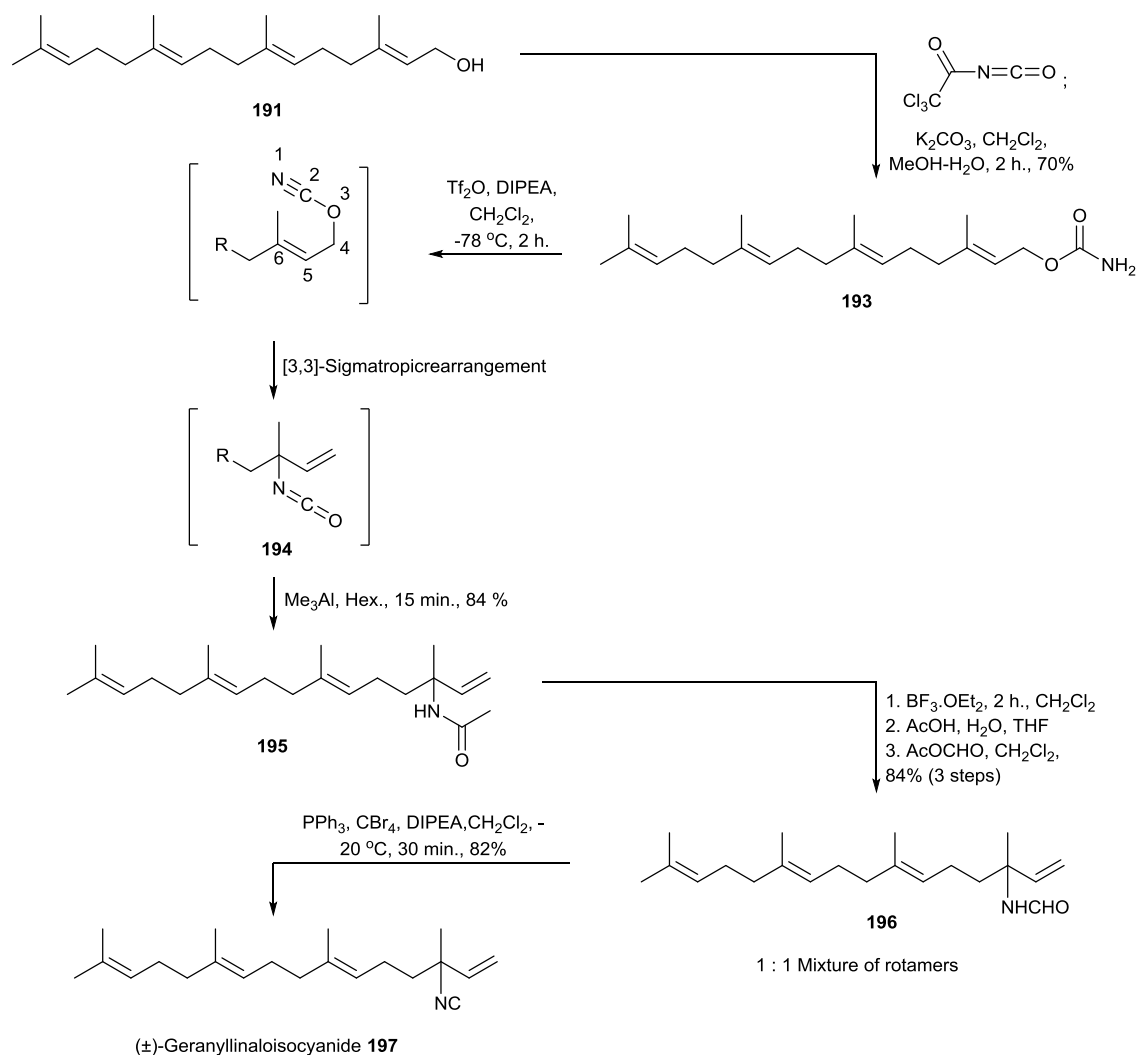


Scheme 17 Srikrishna's synthesis of (-)-2-(isocyano)trachyopsane **192**.<sup>74</sup>

#### 1.1.4. Syntheses of diterpene isocyanide natural products

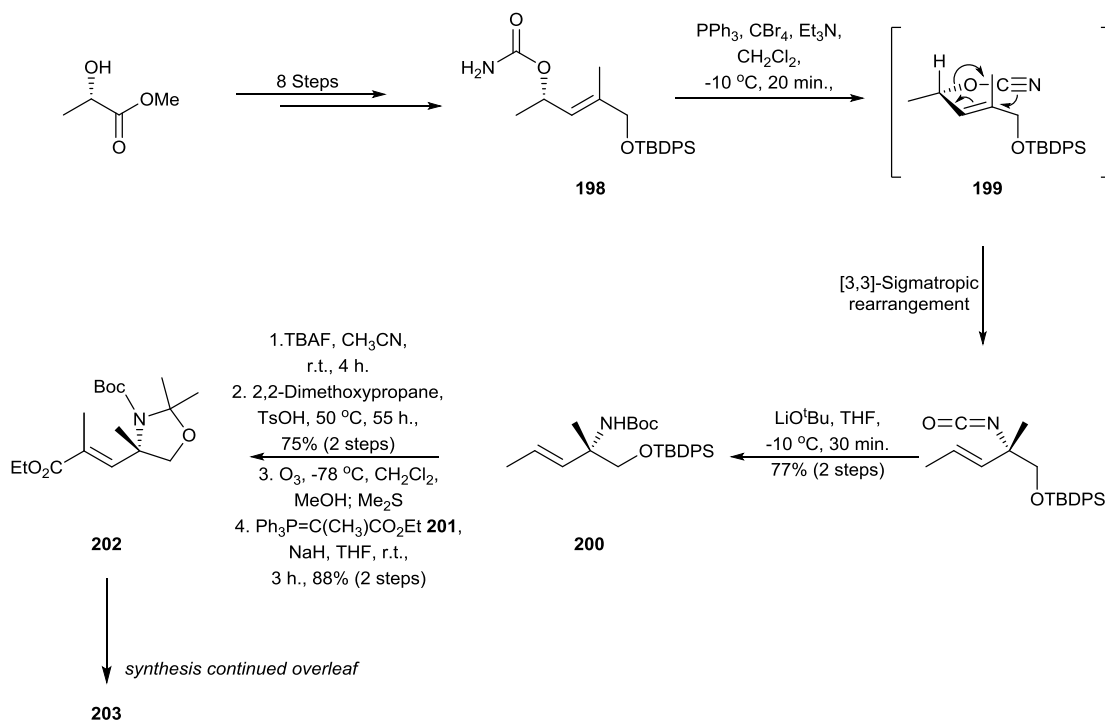
Three different classes of naturally occurring C<sub>20</sub> diterpenoid derived isocyanides isolated from marine sources have been synthesised that contain acyclic, kalihinane, and amphilectane skeletons respectively. (+)-Geranylinaloisocyanide **197** was the first marine diterpene isocyanide isolated in 1974 from the marine sponge *Halichondria sp.* that was shown to be active as an antibiotic against *S. aureus*.<sup>75–77</sup> The first synthesis of (±)-geranylinaloisocyanide was reported by Ichikawa *et al.* (Scheme 18) who demonstrated that treatment of geranylgeraniol **192** with trichloroacetyl isocyanate followed by methoxide afforded carbamate **193**. Treatment of carbamate **194** with triflic anhydride and DIPEA at -78 °C generated a cyanate that underwent a [3,3]-rearrangement reaction *in situ* to afford isocyanate **194**.<sup>78</sup> Treatment of this isocyanate **194** with triethylaluminium chloride resulted in

formation of acetamide **195** that was hydrolysed *via* treatment with  $\text{Et}_3\text{O} \cdot \text{BF}_4$  to give an imino ether intermediate that was hydrolysed with aqueous acetic acid to afford its corresponding amine. Treatment of this amine intermediate with acetic formic anhydride afforded formamide **196**, which was dehydrated to generate the isocyanide functionality of ( $\pm$ )-geranyllinaloisocyanide **197** *via* treatment with  $\text{Ph}_3\text{P}$ ,  $\text{CBr}_4$ , and  $\text{Et}_3\text{N}$ .<sup>78</sup>

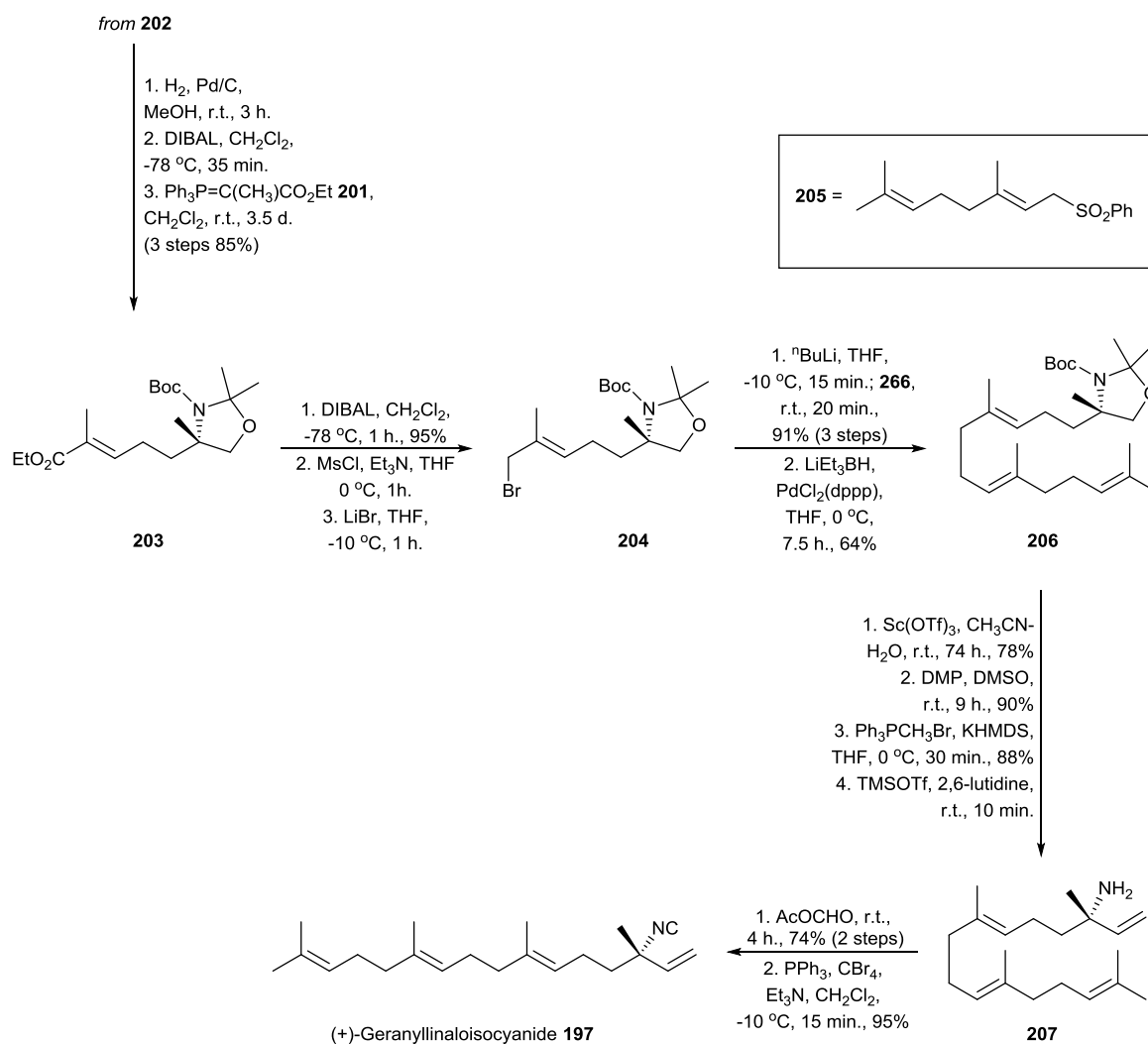
Scheme 18 Ichikawa's synthesis of (±)-geranyllinaloisocyanide **197**.<sup>79</sup>

In 2011, Ichikawa *et al.* subsequently reported a stereoselective synthesis of (+)-geranyllinaloisocyanide **197** (Scheme 19), whereby the quaternary stereocentre of *N*-boc-amide **200** was installed *via* stereoselective [3,3] sigmatropic rearrangement of a cyanate intermediate **199** that was generated *in situ* *via* treatment of carbamate **198** with PPh<sub>3</sub>, CBr<sub>4</sub> and Et<sub>3</sub>N. Subsequent trapping of the isocyanate product with *tert*-butoxide to afford *N*-Boc-

amine **200**. Conversion of *N*-Boc-amine **200** into oxazolidine **202** was then achieved over four steps involving: (i) *O*-TBDPS deprotection; (ii) *N*-*O*-acetal formation; (iii) alkene ozonolysis; (iv) Wittig reaction of the resultant aldehyde group with ylide **201**. Hydrogenation of the alkene functionality of oxazolidine **202** was followed by DIBAL reduction and reaction of the resultant aldehyde with ylide **201** to afford enone **203**. Reduction of ester **202** with DIBAL resulted in formation of an allylic alcohol that was converted into its corresponding mesylate and reacted with LiBr to afford its corresponding allylic bromide **204**. Treatment of bromide **204** with an excess of the carbanion of sulfone **205** afforded a cross-coupled polyene sulfone, whose sulfone group was removed *via* treatment with LiEt<sub>3</sub>BH / PdCl<sub>2</sub>(dppp) to afford polyene **206**. Hydrolysis of the oxazolidine fragment of polyene **206** was followed by alcohol oxidation with DMP to afford an *N*-Boc-aldehyde that underwent a Wittig methylenation reaction, followed by *N*-Boc deprotection using trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine to give polyene amine **207**. This amine **207** was then treated with acetic formic anhydride to afford its corresponding formamide that was dehydrated *via* reaction with PPh<sub>3</sub>, CBr<sub>4</sub> and Et<sub>3</sub>N to afford the isocyanide functionality of (+)-geranylinaloisocyanide **197**. Comparison of the sign of the specific rotation of this synthetic material enabled the configuration of the quaternary stereocentre of the natural product **197** to be assigned as (*S*)- for the first time.<sup>77</sup>







Scheme 19 Ichikawa's synthesis of (+)-geranyllinaloisocyanide **197**.<sup>77</sup>

A range of tricyclic kalihinol isocyanides have been isolated from the sponge species *Acanthella* sp.,<sup>79</sup> which contain either *cis*- or *trans*- fused decalin ring systems that are attached to either tetrahydrofuran, tetrahydropyran or dihydropyran rings. They are thought to be produced by sponges for their antifouling and cytotoxic activities, however they have also been shown to exhibit a range of other useful biological activity. For example, (+)-kalihinol A **3** exhibits antimalarial and antibiotic activity,<sup>80–82</sup> kalihinol F **8** inhibits topoisomerase I in starfish,<sup>83</sup> and (–)-kalihinol Y **208** inhibits the growth of *Bacillus subtilis*, *S. aureus*, *Candida albicans* and displays anthelmintic activity against *Nippostrongylus brasiliensis* (Figure 13).<sup>84,85</sup>

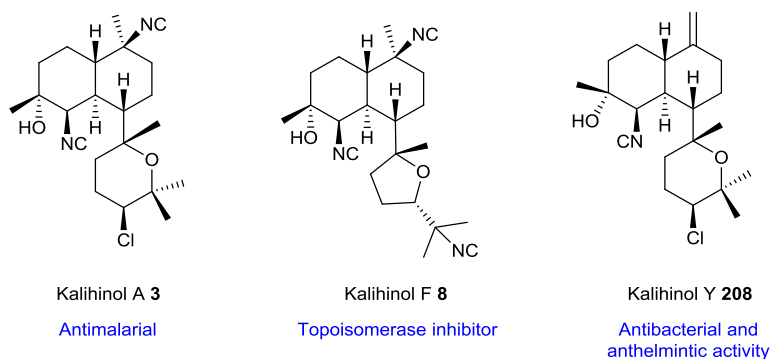
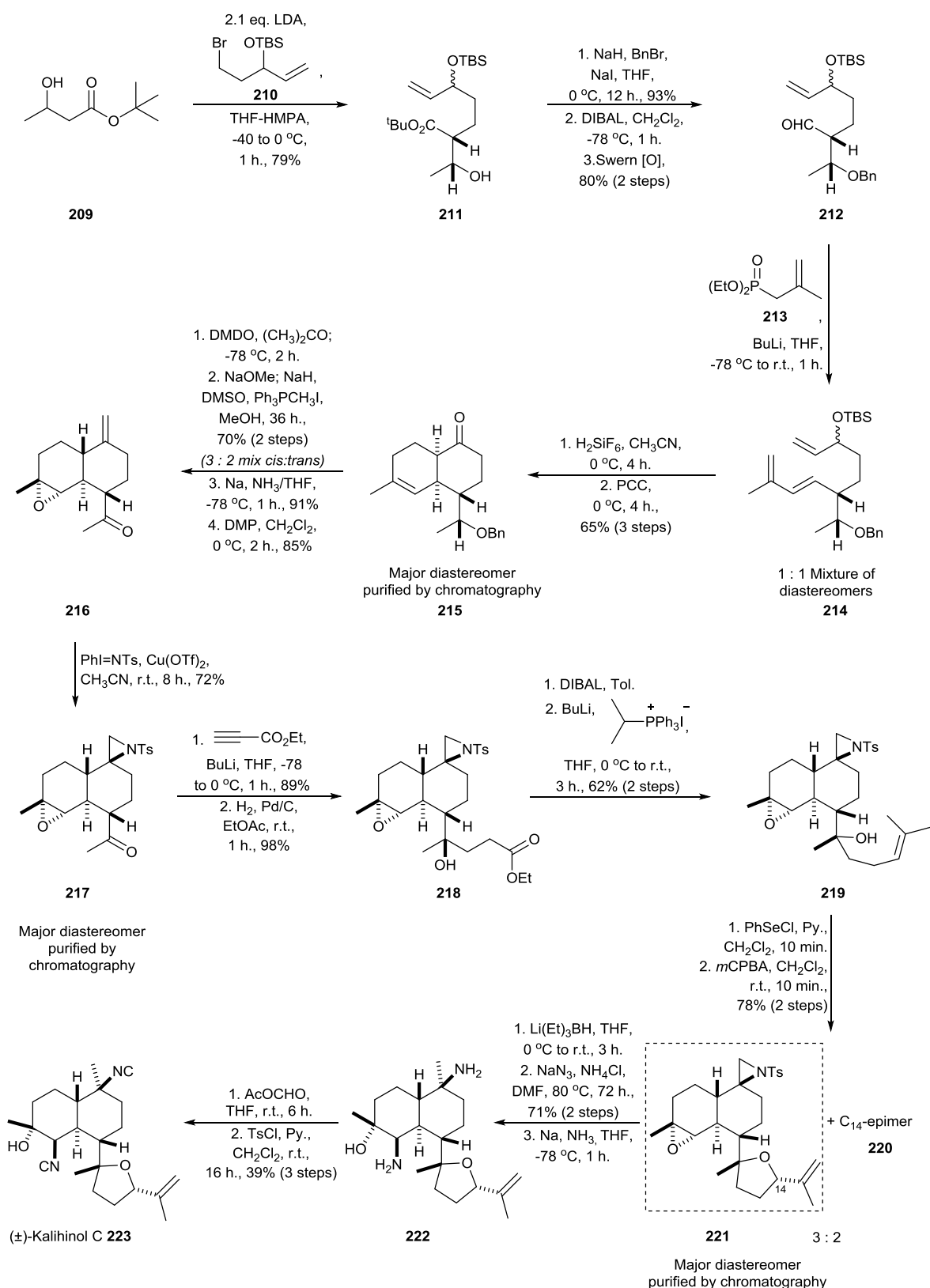


Figure 13 Biological activities of kalihinols A **3**, F **8** and Y **208**.<sup>80–85</sup>

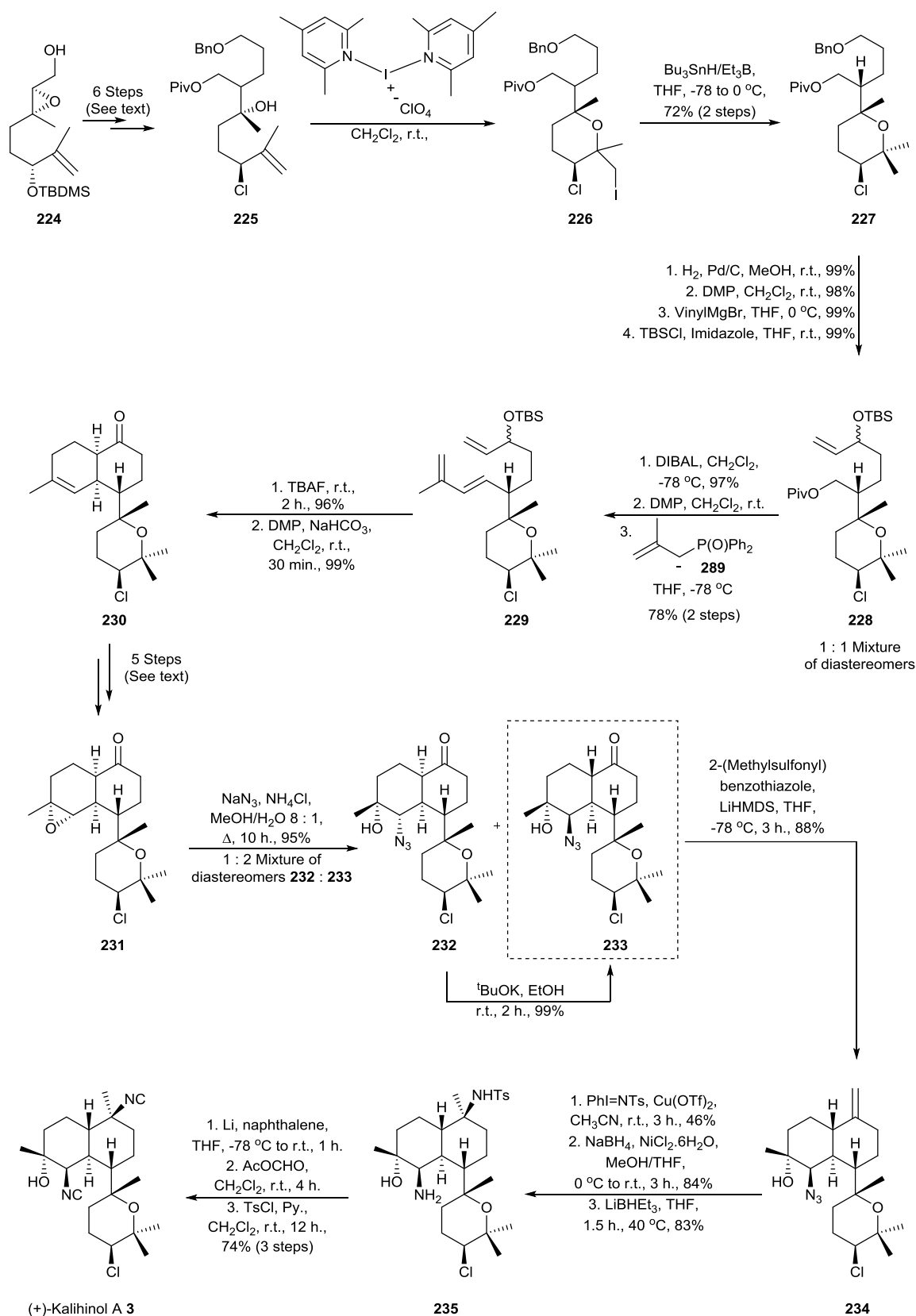
Wood and co-workers have reported the synthesis of ( $\pm$ )-kalihinol C **223** (Scheme 20),<sup>86</sup> whose *cis*-decalin core was assembled *via* an intramolecular Diels-Alder reaction of triene intermediate **214** that was assembled *via* sequential alkylation of the enolate of  $\beta$ -hydroxy ester **209** with alkyl bromide **210**, followed by Horner-Wadsworth-Emmons homologation of aldehyde **212** with the anion of phosphonate **213**. *O*-Silyl deprotection of triene **214** was followed by oxidation of the resultant alcohol with PCC to afford bicyclic ketone **215**. This ketone **215** was then transformed into epoxy-aziridine **217** over five steps, involving: (i) stereoselective epoxidation with dimethyldioxirane (DMDO) to afford an  $\alpha$ -epoxide; (ii) Wittig methylenation of the ketone functionality with concomitant epimerisation resulting in a *trans*-fused decalin ring; (iii) dissolving metal reduction of the *O*-benzyl protecting group; (iv) oxidation of the resultant alcohol to afford alkene **216**; (v) stereoselective aziridination with  $\text{PhI=NTs/Cu(OTf)}_2$  to afford epoxy-aziridine **217** as the major diastereomer. Addition of the lithium anion of ethyl propiolate to the ketone group of epoxy-aziridine **217** gave an alcohol intermediate whose alkyne functionality was fully hydrogenated to afford saturated ester **218**. Reduction of ester **218** with DIBAL afforded a lactol intermediate that was reacted with  $\text{Ph}_3\text{P=CMe}_2$  to afford the isopropylene fragment of alcohol **219**. Seleno-etherification ( $\text{PhSeCl}/m\text{CPBA}$ ) of the alkene bond of **219** resulted in formation of a tetrahydrofuran ring with modest preference (3 : 2) for the desired diastereomer **221** over its  $\text{C}_{14}$ -epimer **220**. After purification by chromatography, treatment of aziridine **221** with  $\text{LiEt}_3\text{BH}$  resulted in formation of a tertiary *N*-tosyl-amino group, with reaction of its epoxy group with ammonium azide then affording an azido-alcohol group with good regio-control. Simultaneous reduction of both the *N*-Tosyl and azido groups of this intermediate was achieved under dissolving metal conditions ( $\text{Na/NH}_3$ ) to afford a *bis*-amine **222** that was *N*-formylated using acetic formic anhydride and dehydrated using  $\text{TsCl}$  in pyridine to afford the *bis*-isocyanide functionality of ( $\pm$ )-kalihinol C **223**.<sup>86</sup>

### Methodology for the synthesis of isocyanide containing natural products

Scheme 20 Wood's synthesis of (±)-kalihinol C **223**.<sup>86</sup>

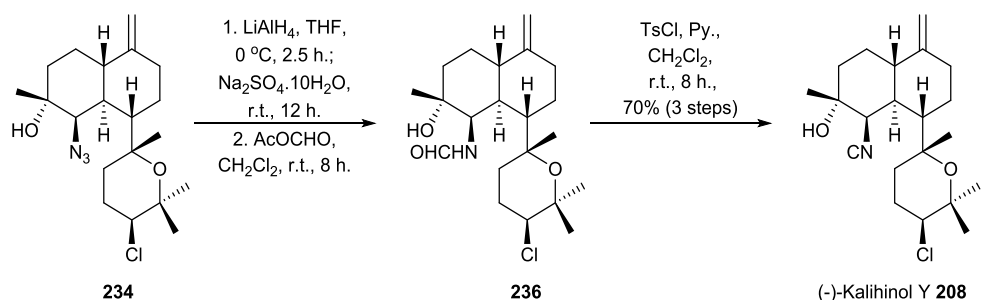
Miyaoka recently reported the synthesis of (+)-kalihinol A **3** (Scheme 21),<sup>81</sup> starting from chiral epoxide **224** that was converted into chloride **225** *via* a series of six reactions involving: (i) regioselective epoxide ring opening using copper catalysed addition of  $\text{BrMg}(\text{CH}_2)_3\text{OBn}$ ; (ii) *bis*-acetylation of the resultant diol; (iii) *O*-silyl deprotection of the allylic alcohol functionality; (iv)  $\text{S}_{\text{N}}2$  allylic substitution of the hydroxyl group with chloride (using  $\text{Cl}_3\text{CCOCCl}_3/\text{Ph}_3\text{P}$ ); (v) reductive deprotection of both acetate protecting groups; (vi) monopivaloylation of the primary alcohol group. Treatment of the resultant alkene **225** with iodonium di-sym-collidine perchlorate resulted in intramolecular iodoetherification to afford an iodotetrahydropyran **226**, whose iodide group was removed *via* treatment with  $\text{Bu}_3\text{SnH}$  and  $\text{Et}_3\text{B}$  to afford tetrahydropyran **227**. The benzyl protecting group of **227** was removed *via* hydrogenolysis and the resulting free alcohol group oxidised to an aldehyde that was reacted with vinylmagnesium bromide and the resulting mixture of epimeric alcohols that were protected as their *O*-TBDMS ethers **228**. The pivaloyl protecting groups of **228** were removed reductively *via* treatment with DIBAL, and the resultant epimeric primary alcohols oxidised to an aldehyde which was reacted with the anion of  $\text{CH}_2=\text{C}(\text{Me})\text{CH}_2\text{P}(\text{O})\text{Ph}_2$  to afford the diene fragment of chloride **229**. Triene **229** was then *O*-silyl deprotected/oxidised to afford an enone that underwent an *endo*-selective intramolecular Diels-Alder reaction to construct the *cis*-decalin core of tricyclic ketone **230**. Attempts to epoxidise the alkene functionality of **230** with *m*CPBA afforded poor selectivity for the desired  $\alpha$ -epoxide, therefore an indirect five-step approach was devised for its synthesis. This involved: (i) reduction of the keto functionality of **230** with  $\text{NaBH}_4$ ; (ii) *O*-*tert* butylsilyl (TBS) protection of the resultant alcohol; (iii) stereoselective epoxidation of the alkene group from the least hindered face to afford the desired  $\alpha$ -epoxide; (iv) *O*-silyl deprotection; (v) oxidation of the alcohol group to generate epoxy-ketone **231**. Epoxy ketone **231** was then treated with sodium azide in dimethyl formamide (DMF) resulting in regioselective epoxide ring-opening to afford a mixture of *trans*-decalin **232** and *cis*-decalin **233**. *Trans*-decalin **233** was then treated with the anion of 2-(methylsulfonyl)benzothiazole to introduce the *exo*-methylene fragment of azide **234** that was diastereoselectively aziridinated using  $\text{PhI}=\text{NTs}/\text{Cu}(\text{OTf})_2$  to afford an aziridine. The azide and aziridine functionalities of this intermediate were then reduced using  $\text{NaBH}_4/\text{NiCl}_2$  and  $\text{LiBHET}_3$  respectively, to afford mono-*N*-tosylated *bis*-amine **235**. The *N*-tosyl group of **236** was then removed reductively *via* treatment with lithium in naphthalene/THF, with both amino groups then being *N*-formylated with acetic formic anhydride, and the resultant *N*-formamide groups dehydrated ( $\text{TsCl}/\text{pyridine}$ ) to afford the *bis*-isocyanide functionalities of (+)-kalihinol A **3**.<sup>81</sup>

Methodology for the synthesis of isocyanide containing natural products



Scheme 21 Miyaoka's synthesis of (+)-kalihinol A 3.<sup>81</sup>

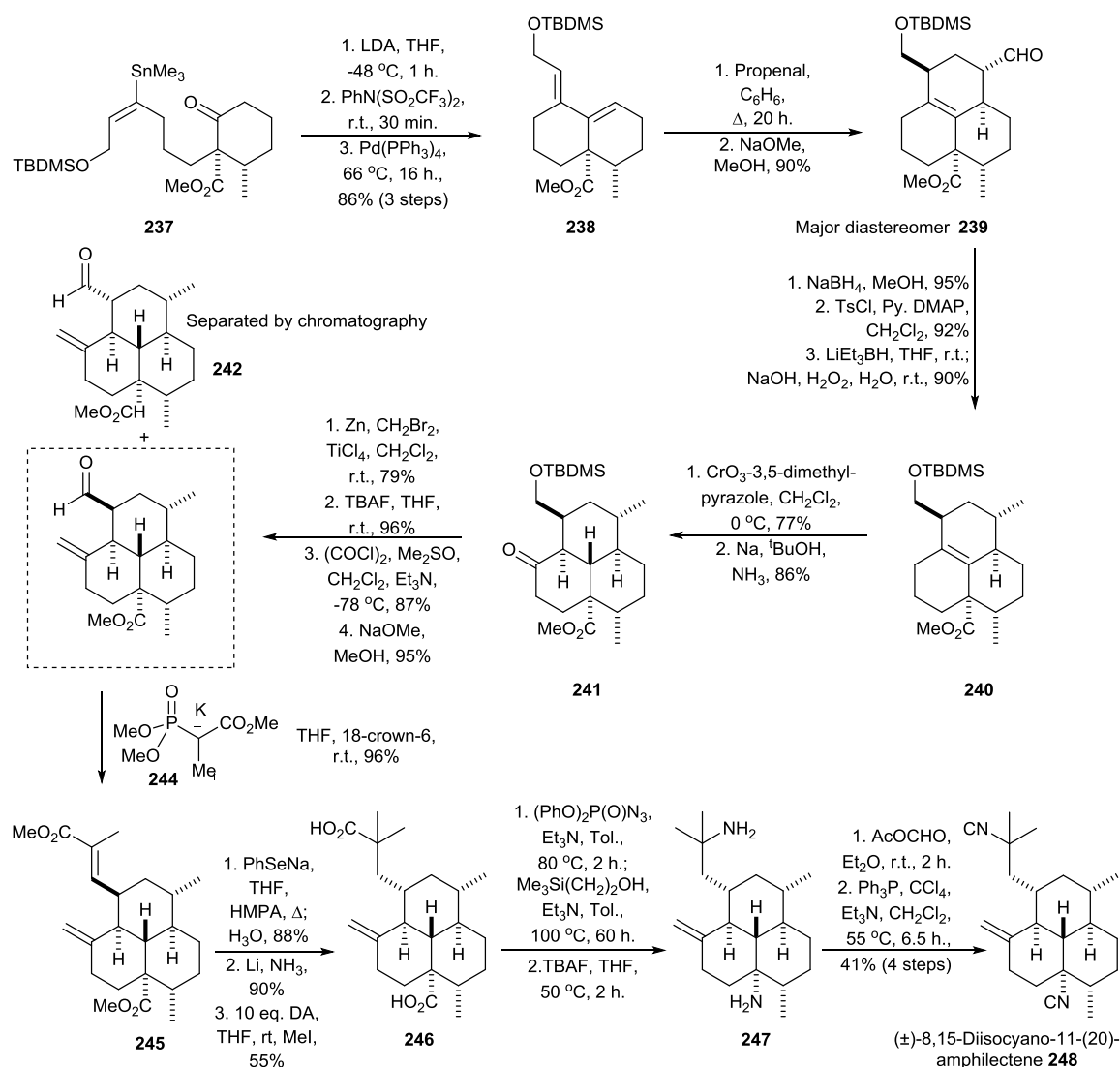
Miyaoka *et al.* also reported the synthesis of (-)-kalihinol Y **208** in 2012 (Scheme 22),<sup>87</sup> which has been shown to demonstrate antibacterial activity by inhibiting folate acid biosynthesis,<sup>79,88</sup> as well as anthelmintic activity.<sup>84</sup> Azide **234**, prepared in the previous synthesis of Kalihinol A **3** (see scheme 21), was reduced into its corresponding amine using LiAlH<sub>4</sub>, *N*-formylated using acetic formic anhydride to afford formamide **236** and dehydrated *via* treatment with TsCl and pyridine to afford (-)-kalihinol Y **208**.<sup>87</sup>



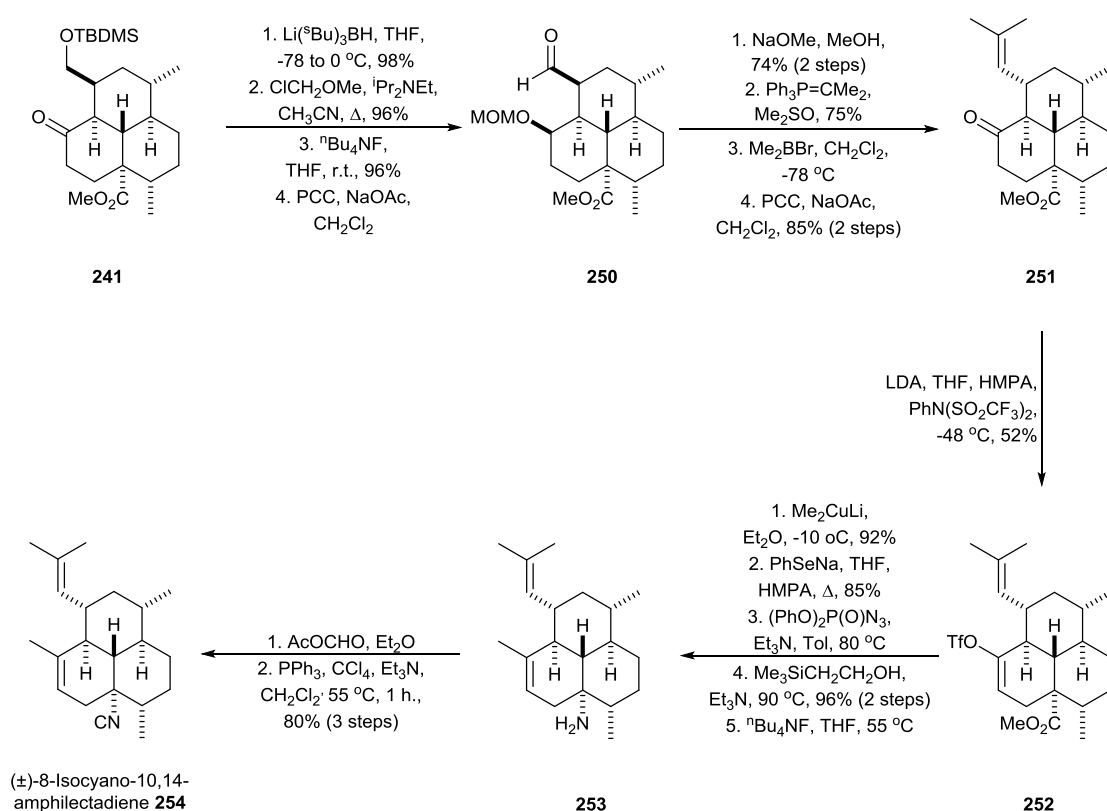
Scheme 22 Miyaoka's synthesis of (-)-kalihinol Y **208**.<sup>87</sup>

The amphilectane derived isocyanides are a class of diterpenoid natural products derived from fused tricyclic or tetracyclic cyclohexyl skeletons that contain one or more quaternary isocyanide groups, that have been shown to exhibit antibiotic and antimalarial activity.<sup>89–91</sup> (±)-8,15-Diisocyano-11 (20)-amphilectene **248**, originally isolated by ethanolic extraction of a freeze dried sponge, is a fused tricyclic amphilectane diterpenoid that inhibits the growth of both *S. aureus* and *B. subtilis*.<sup>92</sup> Its total synthesis has been reported by Piers and Llinas-Brunet (scheme 23),<sup>93,94</sup> using a strategy which commenced with the conversion of ketone **237** into its corresponding enol triflate that then underwent an intramolecular Pd(0)-catalysed cross coupling reaction to afford the bicyclic diene ester **238**. This ester **238** underwent a thermal intermolecular Diels-Alder reaction with propenal to introduce the third cyclohexenyl ring of aldehyde **239**, which was obtained as a major diastereomeric product after base catalysed epimerisation. After purification by chromatography, aldehyde **239** was reduced with NaBH<sub>4</sub> to afford an alcohol that was then tosylated and reduced out using LiEt<sub>3</sub>BH to introduce the second methyl group of ester **240**. Ester **240** was then treated with CrO<sub>3</sub>-3,5-dimethylpyrazole resulting in allylic oxidation to afford an enone that underwent conjugate reduction on treatment with Na/NH<sub>3</sub> to produce a ketone **241** with a new *trans*-fused ring junction. This ketone **241** was then reacted with zinc dust, CH<sub>2</sub>Br<sub>2</sub> and TiCl<sub>4</sub> to introduce an *exo*-methylene group, with *O*-silyl ether deprotection using TBAF affording an alcohol that was oxidised with Swern's reagent to afford an aldehyde whose α-stereocentre was epimerised *via* treatment

with sodium methoxide. The resultant major aldehyde diastereomer **243** was purified by chromatography and subjected to a Horner-Wadsworth-Emmons reaction with the potassium salt of trimethyl 2-phosphonopropionate **244** which gave a 7 : 2 mixture of geometric (*E*-)/(*Z*-) isomers that were separated by chromatography. Treatment of the major *bis*-ester **245** with sodium benzeneselenoate resulted in hydrolysis of both ester groups to afford a diacid intermediate whose enone functionality was then reduced *via* treatment with Li/NH<sub>3</sub>. Treatment of the resultant saturated diacid with excess LDA, followed by  $\alpha$ -alkylation of the resultant trianion with methyl iodide, introduced the geminal dimethyl group of diacid **246**. Standard Curtius rearrangement methodology ((i) DPPA; (ii) 2-TMS-EtOH; (iii) TBAF) was then used to convert both diacid groups of **246** into the diamino functionalities of **247** which was then treated sequentially with acetic formic anhydride and Ph<sub>3</sub>P, CCl<sub>4</sub> and Et<sub>3</sub>N to afford the *bis*-isocyanide functionality of ( $\pm$ )-8,15-diisocyano-11-(20)-amphilectene **248**.<sup>93,94</sup>


 Scheme 23 Piers' synthesis of ( $\pm$ )-8,15-diisocyano-11-(20)-amphilectene **248**.<sup>93,94</sup>

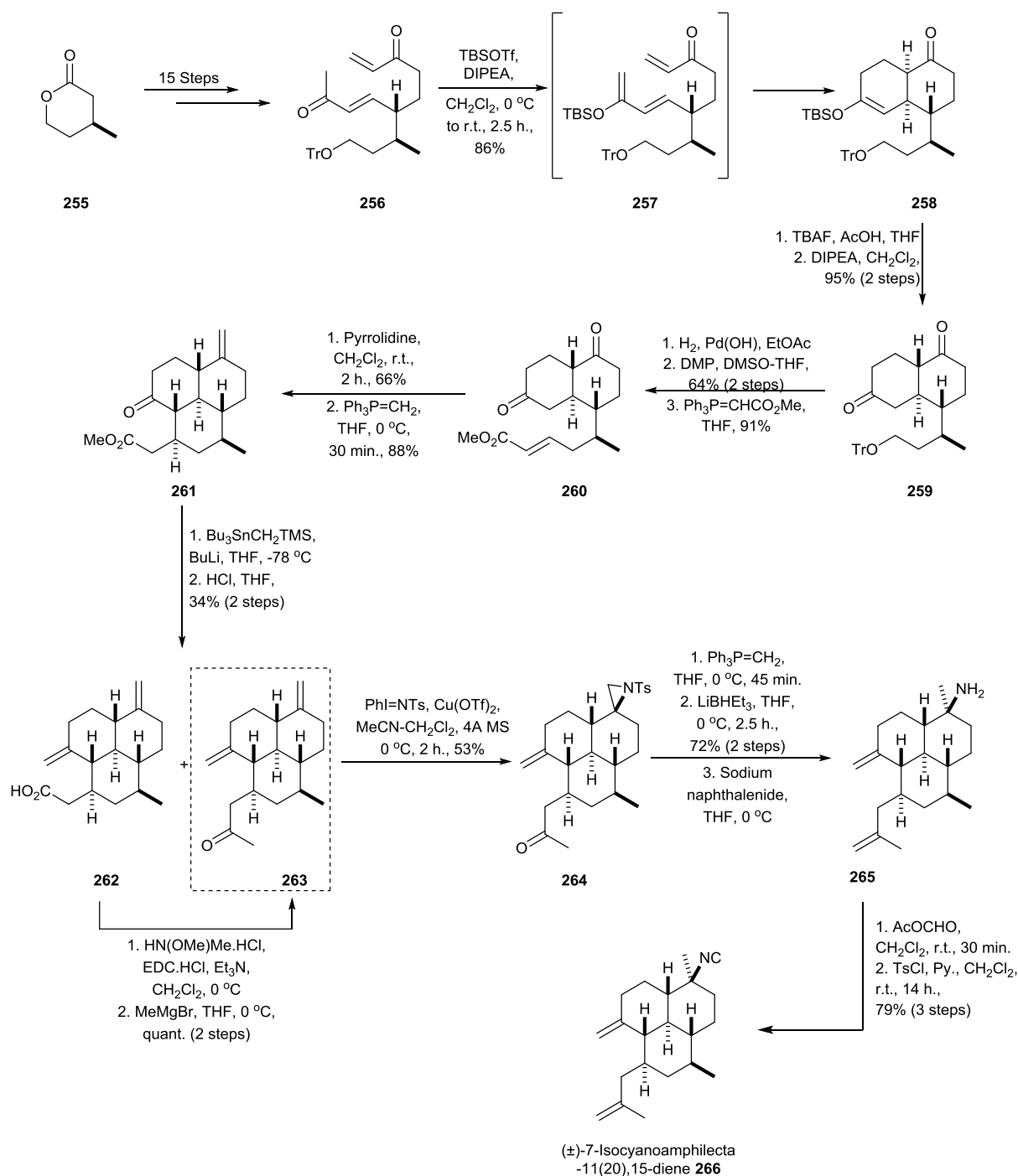
Piers and Romero also reported the synthesis of (±)-8-isocyano-10,14-amphilectadiene **254** (Scheme 24),<sup>95</sup> which was originally isolated from the marine sponge *Hymeniacidon amphilecta* and shown to inhibit the growth of *S. aureus* and *B. subtilis*.<sup>89,90</sup> The previously reported ketone **241** was reduced stereoselectively with  $\text{Li}(\text{}^t\text{Bu})_3\text{BH}$  and the resulting hydroxyl group protected as its MOM ether, followed by *O*-silyl deprotection to afford an alcohol group that was oxidised with PCC to afford aldehyde **250**. Epimerisation of the  $\alpha$ -stereocentre of aldehyde **250** with NaOMe was followed by Wittig reaction with  $\text{Ph}_3\text{P}=\text{C}(\text{Me})_2$ , MOM protecting group removal and oxidation of the resultant secondary alcohol with PCC to afford ketone **251**. Reaction of the lithium enolate of ketone **251** with phenyltrifluoromethanesulfonimide in HMPA afforded a vinyl triflate **252** that was treated with an excess of  $\text{Me}_2\text{CuLi}$  to give a diene. This diene was subsequently hydrolysed into its corresponding acid that was then converted into (±)-8-isocyano-10,14-amphilectadiene **254** over five steps (*via* amine **253**) using the Curtius rearrangement methodology developed previously for the synthesis of (±)-8,15-diisocyano-11-(20)-amphilectacene **248** (see scheme 23).<sup>95</sup>

Scheme 24 Piers' synthesis of (±)-8-isocyano-10,14-amphilectadiene **254**.<sup>95</sup>



Miyaoka and co-workers have reported the total synthesis of (±)-7-isocyanoamphilecta-11(20),15-diene **266** (Scheme 25),<sup>96</sup> an antimalarial diterpenoid originally isolated from the tropical marine sponge *Cymbastela hooperi*.<sup>91</sup> Lactone **255** was converted into *bis*-enone **256** in 15 steps, which was then treated with TBSOTf and DIPEA to regioselectively afford a silyl enol ether **257** that underwent an intramolecular *endo*-selective Diels-Alder reaction to afford the *cis*-decalin ring system of ketone **258**. This ketone **258** was then treated with TBAF to afford a diketone that was epimerised *via* treatment with DIPEA to afford the *trans*-decalin framework of diketone **259**. The trityl group of *trans*-decalin **259** was then removed *via* hydrogenation to give a primary alcohol that was oxidised with DMP to afford an aldehyde that underwent a Wittig homologation reaction with  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$  to afford  $\alpha,\beta$ -unsaturated ester **260**.  $\alpha,\beta$ -Unsaturated ester **260** was treated with pyrrolidine to afford an enamine that underwent a stereoselective intramolecular conjugate addition reaction to give the thermodynamically more stable all-*trans* tricyclic perhydrophenalene ring system of a diketone that was mono-olefinated with  $\text{Ph}_3\text{P}=\text{CH}_2$  to afford enone **261**. The sterically hindered ketone group of **261** was then methylenated *via* a Peterson olefination reaction using  $\text{Me}_3\text{SiCH}_2\text{Li}$  to give a mixture of carboxylic acid **262** and ketone **263**, with acid **262** subsequently being recycled into ketone **263** *via* a two-step procedure involving Weinreb amide formation and reaction with  $\text{MeMgBr}$ . The least-hindered alkene functionality of ketone **263** was then aziridinated *via* treatment with  $\text{PhI}=\text{NTs}$  to afford *N*-tosyl-aziridine **264**, whose ketone group was methylenated ( $\text{Ph}_3\text{P}=\text{CH}_2$ ), its aziridine ring regioselectively reduced with  $\text{LiBHET}_3$  and its *N*-tosyl group removed using sodium naphthalenide in THF to afford amine **265**. Amine **265** was then *N*-formylated *via* treatment with acetic formic anhydride and subsequently dehydrated using  $\text{TsCl}$  in the presence of pyridine to afford the isocyanide functionality of (±)-7-isocyanoamphilecta-11(20),15-diene **266**.<sup>96</sup>

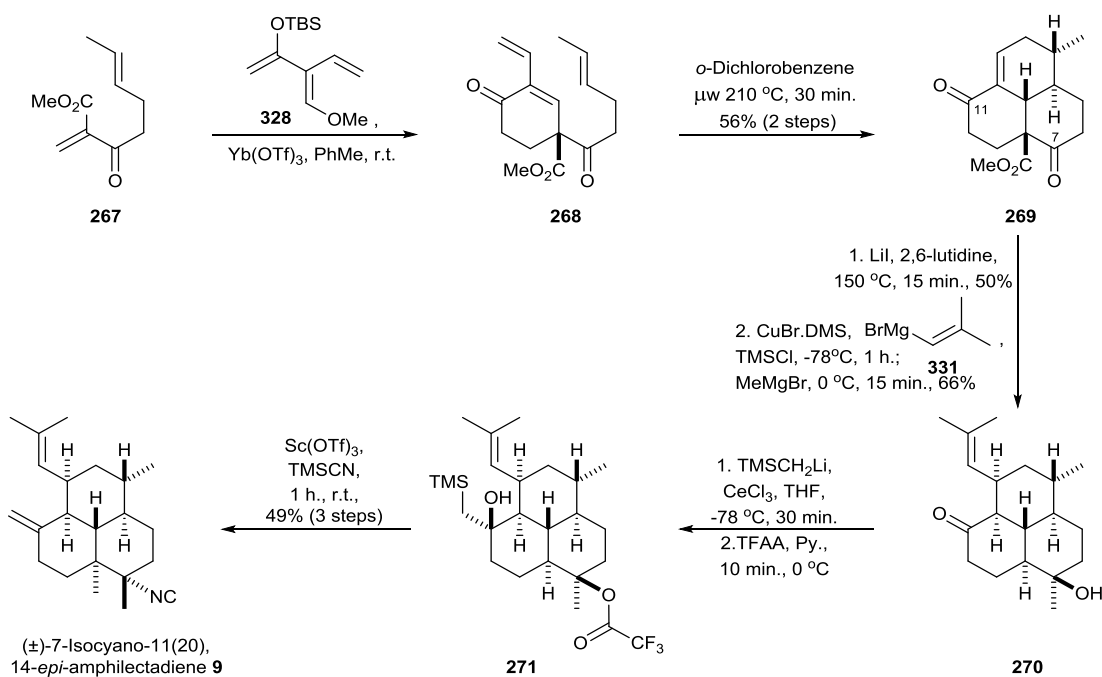
Methodology for the synthesis of isocyanide containing natural products



Scheme 25 Miyaoka's synthesis of (±)-7-isocyanoamphilecta-11(20),15-diene 266.<sup>96</sup>

Pronin and Shenvi have recently reported an elegant synthesis of (±)-7-isocyano-11(20),14-*epi*-amphilectadiene **9** (Scheme 26),<sup>97</sup> which has good antimalarial activity towards chloroquine-resistant *P. falciparum*.<sup>91</sup> They employed an elegant tandem Diels-Alder strategy to rapidly assemble its tricyclic core, employing a dilute solution of dienyl-ester **267** as a substrate for a Yb(OTf)<sub>3</sub> catalysed intermolecular Diels-Alder reaction with a Danishefsky's diene-like dendralene that gave cross conjugated enone **268**, which then underwent an intramolecular thermal Diels-Alder cycloaddition upon microwave heating in *ortho*-dichlorobenzene to afford

the *cis*-fused enone **369**. This tricycle **369** was treated with Lil and 2,6-lutidine, resulting in a Krapcho decarboxylation reaction that removed the bridgehead methyl ester to afford a tricyclic enedione with the correct *trans*-fused ring junction. Conjugate addition of the cuprate of Grignard reagent to the resultant enone in the presence of TMSCl afforded an intermediary silyl enol ether whose C<sub>7</sub>-ketone group was reacted with MeMgBr *in situ* to afford hydroxy-ketone **270** after work-up. Ketone **270** was then reacted with TMSCH<sub>2</sub>Li in the presence of CeCl<sub>3</sub>, resulting in nucleophilic addition of a TMSiCH<sub>2</sub> group to the C<sub>4</sub>-ketone group, with subsequent exposure of the alcohol functionality to TFAA selectively affording monotrifluoroacetate **271**. Ionisation of trifluoroacetate **271** using the weak oxophilic Lewis acid Sc(OTf)<sub>3</sub> in the presence of 15 equivalents of TMSCN resulted in stereoselective nucleophilic displacement of the trifluoroacetate group as well as elimination of the hydroxyl and silyl groups, to install the isocyanide and alkene functionalities of (±)-7-isocyano-11(20),14-*epi*-amphilectadiene **9**.<sup>97</sup>

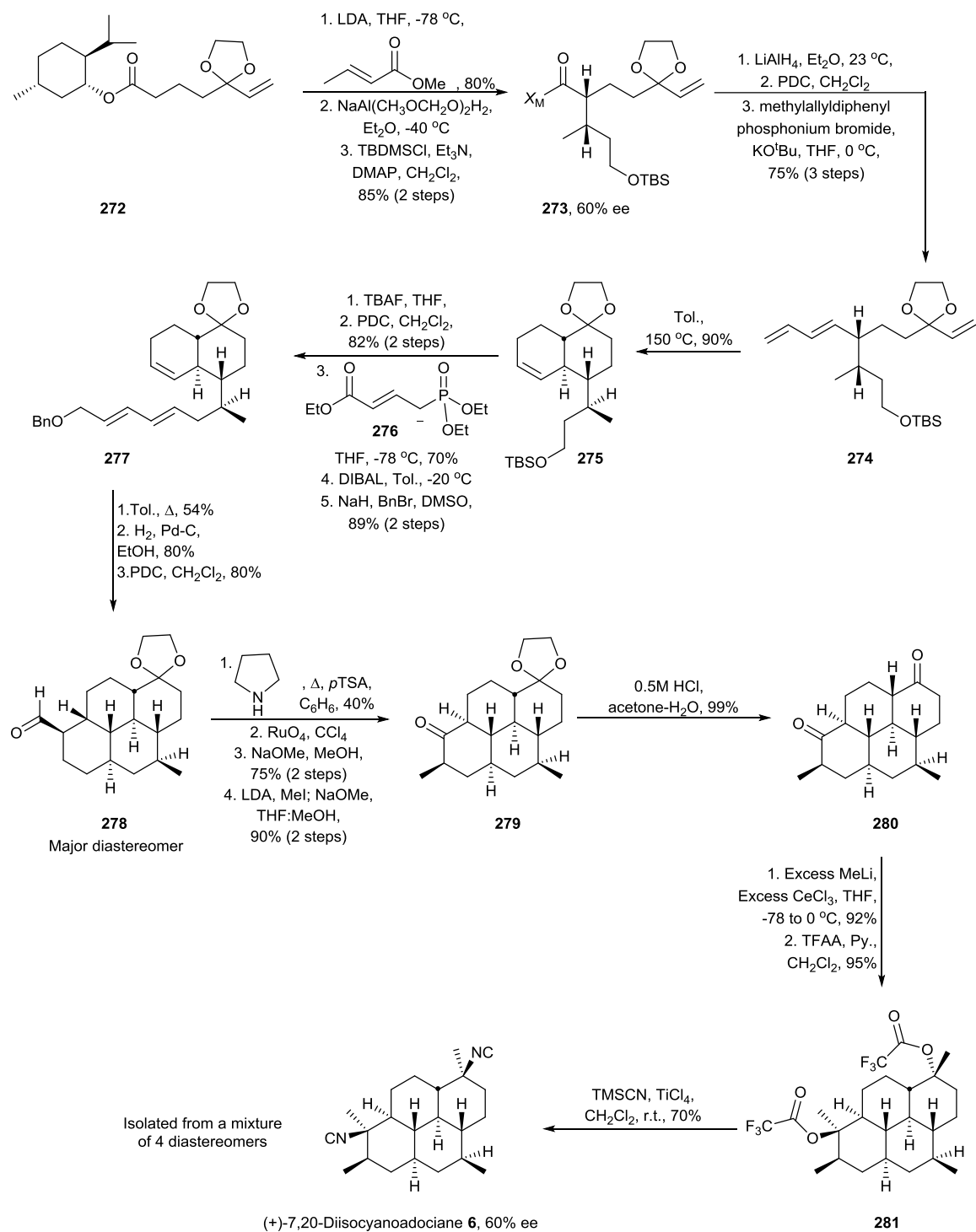


Scheme 26 Shenvi's synthesis of (±)-7-isocyano-11(20),14-*epi*-amphilectadiene **9**.<sup>97</sup>

In 1987, Corey and Magriotis reported the synthesis and configuration of 7,20-diisocyanoadociane **6** (scheme 27), a marine natural product isolated from *Adocia* sp., isolated from a freeze-dried sponge,<sup>98</sup> which has been shown to have anti-malarial activity. For its synthesis,<sup>99</sup> the enolate of chiral ester **272** underwent stereoselective addition to methyl

crotonate to afford a *bis*-ester intermediate (60% ee) whose methyl ester fragment was selectively reduced to afford an alcohol group that was protected as its *O*-TBS ether (7,20-Scheme 27). The menthyl ester fragment of **273** was then reduced with LiAlH<sub>4</sub> to afford an alcohol that was oxidised to its corresponding aldehyde using PDC, which then underwent a Wittig reaction with the anion of methylallyldiphenyl phosphonium bromide to afford the diene fragment of **274**. Heating triene **274** in toluene at 150 °C resulted in a thermal intramolecular Diels-Alder reaction to afford *trans*-fused decalin **275** as a major diastereomer. The *O*-TBS group of **275** was deprotected and the resulting hydroxyl group oxidised to afford an aldehyde that was subjected to a Horner-Wadsworth-Emmons reaction with triethyl lithio-phosphono-(*E*)-crotonate **276** to give a triene ester intermediate that was reduced with LiAlH<sub>4</sub> to afford a primary alcohol that was then *O*-benzyl protected to give triene **277**. Subsequent heating of triene **277** in toluene at 185 °C resulted in a second thermal intramolecular Diels-Alder reaction to assemble a tetracyclic ketal with a newly formed *cis*-fused ring junction. Treatment of this ketal with Pd/C and H<sub>2</sub> resulted in hydrogenation of its alkene bond and cleavage of its *O*-benzyl group, followed by oxidation of the resultant primary alcohol to afford aldehyde **278** as a major diastereomer. Reaction of aldehyde **278** with pyrrolidine in benzene in the presence of *p*TSA afforded an enamine that was oxidatively cleaved *via* treatment with RuO<sub>4</sub> to afford a ketone group. The  $\alpha$ -stereocentre of this ketone was then epimerised under thermodynamic control *via* treatment with NaOMe to give an all *trans*-ketone, whose enolate was methylated *via* treatment with LDA and excess MeI to afford ketone **279**. The ketal group of ketone **279** was then hydrolysed and the resultant diketone **280** treated with excess MeLi to afford a *bis-tert*-diol that was acylated to afford its corresponding *bis*-trifluoroacetate **281** *via* treatment with TFAA/pyridine. *Bis*-trifluoroacetate **281** was then treated with TMSCN and TiCl<sub>4</sub>, directly affording a mixture of four *bis*-isocyanide diastereomers that were separated by chromatography, thus allowing the desired scalemic natural product (+)-7,20-diisocyanoadociene **6** (60% ee) to be isolated after chromatographic purification.<sup>99</sup> More recently Miyaoka and co-workers have reported a multi-step synthesis of Corey's diketone intermediate **280** which represents a formal synthesis of (+)-7,20-diisocyanoadociene **6**.<sup>100</sup> Additionally, Mander<sup>101</sup> has published a formal synthesis of (+)-diisocyanoadociene **6** involving synthesis of diamine that had previously been shown as a viable intermediate for its synthesis by Garson and co-workers.<sup>8</sup>

Methodology for the synthesis of isocyanide containing natural products



Scheme 27 Corey's synthesis of (+)-7,20-diisocyanoadociane **6**.<sup>99</sup>

### 1.3 Syntheses of isocyanides derived from L-tyrosine or L-tryptophan

A range of isocyanide derived natural products derived from the  $\alpha$ -amino acids L-tyrosine and L-tryptophan have been isolated that may be classified into three categories: (i) Xanthocillins isolated from terrestrial and marine sources that contain a *bis*-vinyl isocyanide core; (ii) Terrestrial cyclopentyl isocyanides that contain dermadin or trichoviridin skeletons isolated from soil bacteria; (iii) Monoterpenoid indole alkaloid isocyanides such as the hapalindoles, fischerindoles, welwitindolinones and ambiguines that have been isolated from algae and cyanobacteria (Figure 14).<sup>2</sup>

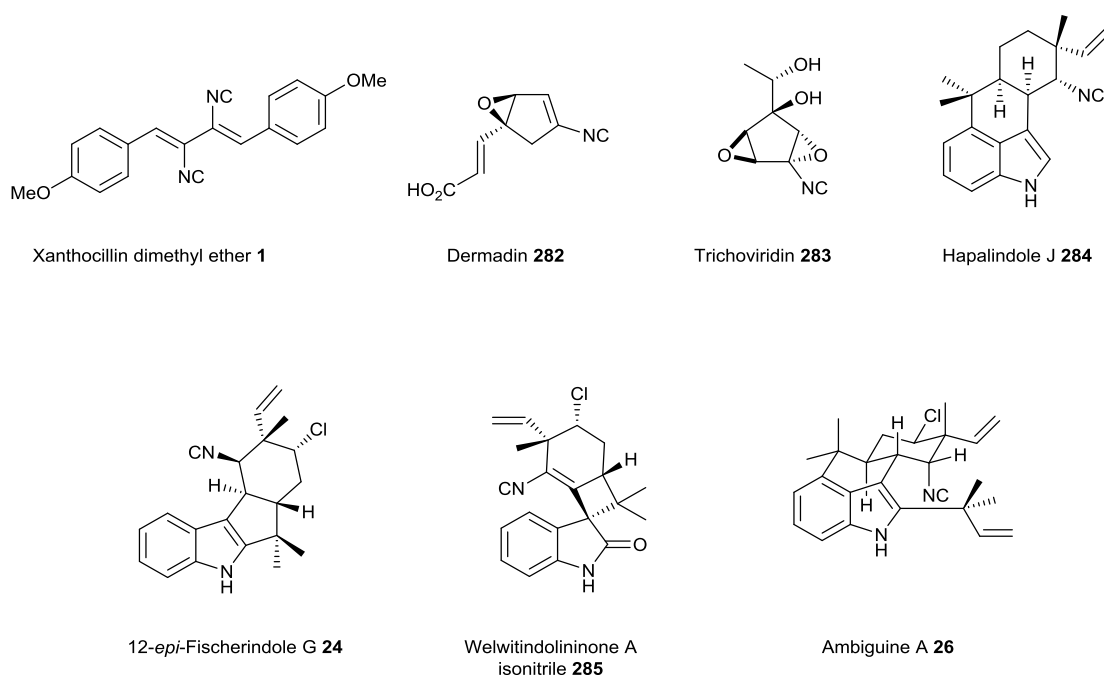


Figure 14 A representative range of L-tyrosine and L-indole derived isocyanide natural products.<sup>2</sup>

#### 1.1.5. Xanthocillin type isocyanides

Xanthocillin dimethyl ether **1**, the first known natural isocyanide, was first isolated from *Penicillium notatum* in 1957,<sup>5</sup> with structural variants subsequently having been reported from a range of terrestrial and marine sources<sup>102</sup> that have been shown to demonstrate a wide range of biological activity, including antibiotic activity,<sup>103</sup> antiviral activity,<sup>104</sup> antifungal activity,<sup>105</sup> and as an agonist of thrombopoietin receptor (Figure 15).<sup>106</sup>

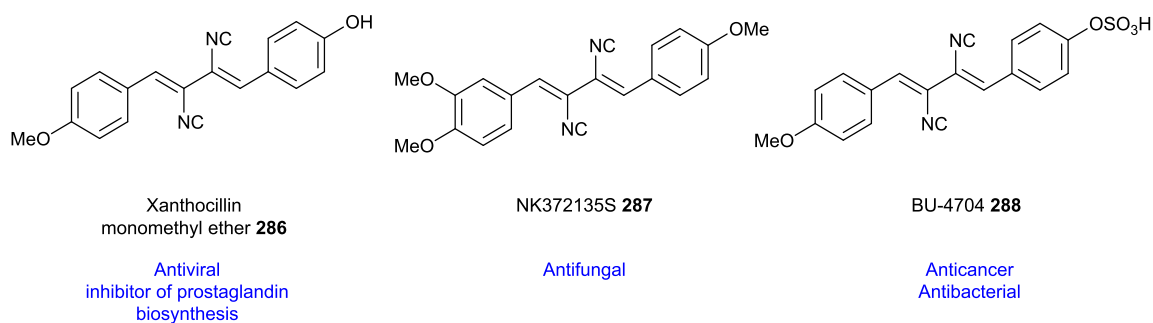
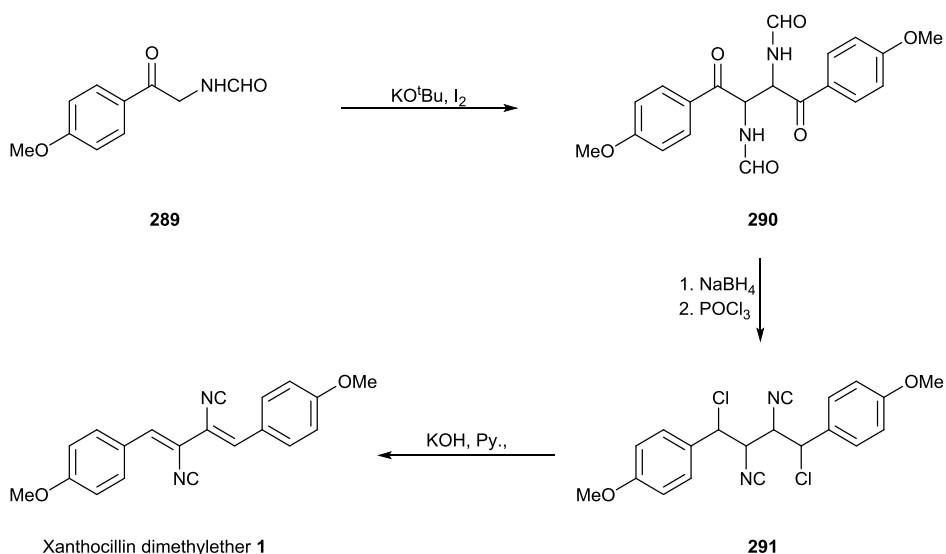


Figure 15 Biologically active xanthocillin derivatives **286-288**.<sup>96,99,107,108</sup>

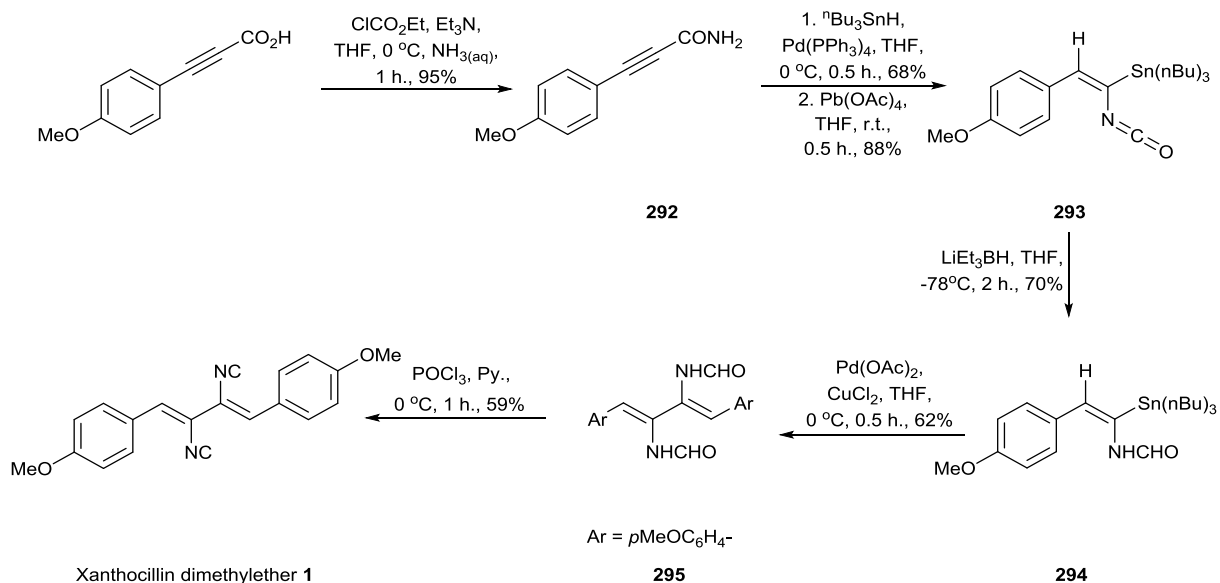
For their synthesis of Xanthocillin dimethyl ether **1** (Scheme 28),<sup>109</sup> Hagedorn *et al.* generated the enolate of 4-methoxy- $\omega$ -formylaminoacetophenone **289** using potassium *tert*-butoxide, which on reaction with iodine resulted in oxidative dimerisation to afford *bis*-formamide **290**. Reduction of diketone **290** with NaBH<sub>4</sub> generated a 1,4-diol that was treated with POCl<sub>3</sub> to generate  $\beta,\beta'$ -dichlorodiisocyanide **291**, which underwent *bis*-E1cB elimination on treatment with KOH and pyridine to afford xanthocillin dimethyl ether **1**.<sup>109,110</sup>



Scheme 28 Hagedorn's synthesis of xanthocillin dimethyl ether **1**.<sup>109,110</sup>

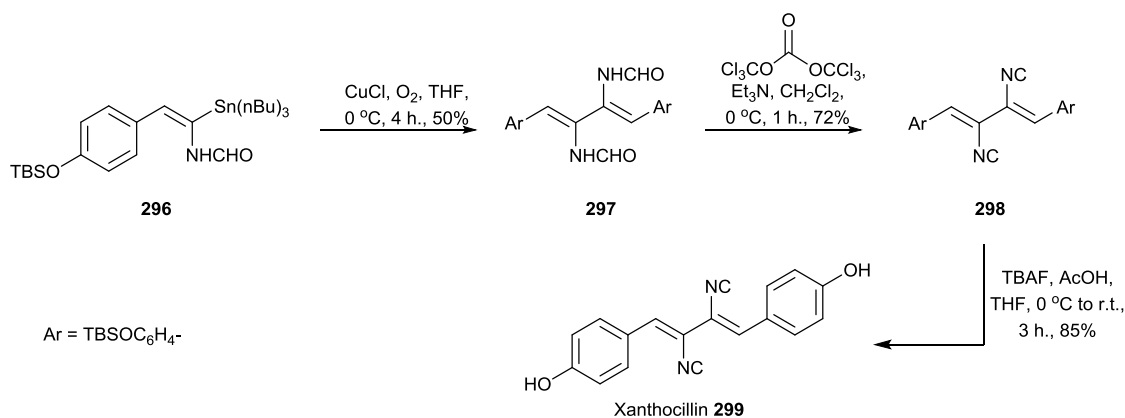
Yamaguchi and Tatsuta have developed an efficient synthesis of xanthocillin dimethylether **1** (Scheme 29),<sup>104</sup> involving hydrostannylation of propiolic acid **292** followed by an oxidative Baumgarten rearrangement to afford isocyanate **293** that was then reduced with LiBH<sub>4</sub> to afford formamide **294** via treatment with LiEt<sub>3</sub>BH. Two equivalents of (*E*)-vinyl-stannane **294** were then homocoupled using a modified Stille reaction that employed a mixed palladium(II)-copper(II) catalytic system to generate (*Z,Z*)-*bis*-formamide **295** in 62% yield. The final step of

the synthesis involved dehydration of (*Z,Z*)-*bis*-formamide **295** using POCl<sub>3</sub> in pyridine to generate the *bis*-isocyanide functionality of xanthocillin dimethylether **1** in 59% yield.<sup>104</sup>



Scheme 29 Tatsuta's synthesis of xanthocillin dimethylether **1**.<sup>104</sup>

Xanthocillin **299** has also been shown to have agonist activity against thrombopoietin receptors in a human leukaemia cell line,<sup>106</sup> with Tatsuta and co-worker preparing analogues (Scheme 30) *via* a modification of their original xanthocillin dimethyl ether **1** synthesis (See scheme 43) involving copper catalysed oxidative coupling of an *O*-TBS-protected vinyl-stannane **296** to afford *bis*-formamide **297**. Generation of the *bis*-isocyanide functionality of **298** was achieved *via* treatment of *bis*-formamide **297** with triphosgene, followed by deprotection of the *O*-silyl groups of **298** with TBAF to afford xanthocillin **299**.<sup>106</sup>



Scheme 30 Synthesis of xanthocillin **299**.<sup>106</sup>



### 1.1.6. Syntheses of cyclopentanoid isocyanides

A series of fungal cyclopentyl derived antibiotics produced by the genus *Trichoderma hamatum* have been isolated from soil cultures,<sup>17,111–115</sup> whose antibiotic activity effectively inhibit the growth of rumen bacteria.<sup>116</sup> These naturally occurring cyclopentyl isocyanides are generally unstable and are difficult to isolate and handle, and are known to inhibit tyrosinase enzymes responsible for melanin biosynthesis as well as acting as inhibitors of multidrug transporters (Figure 16).

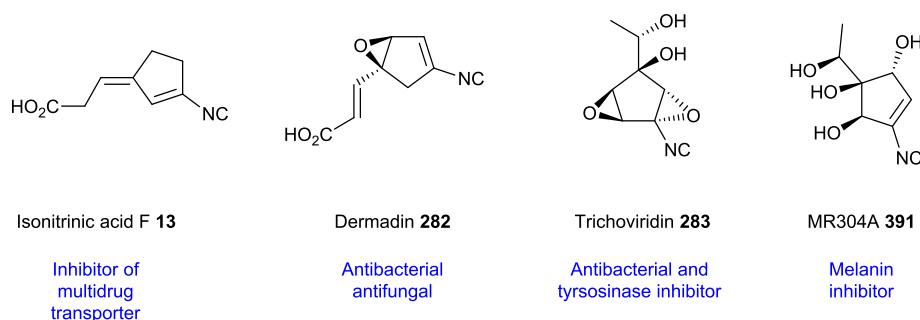
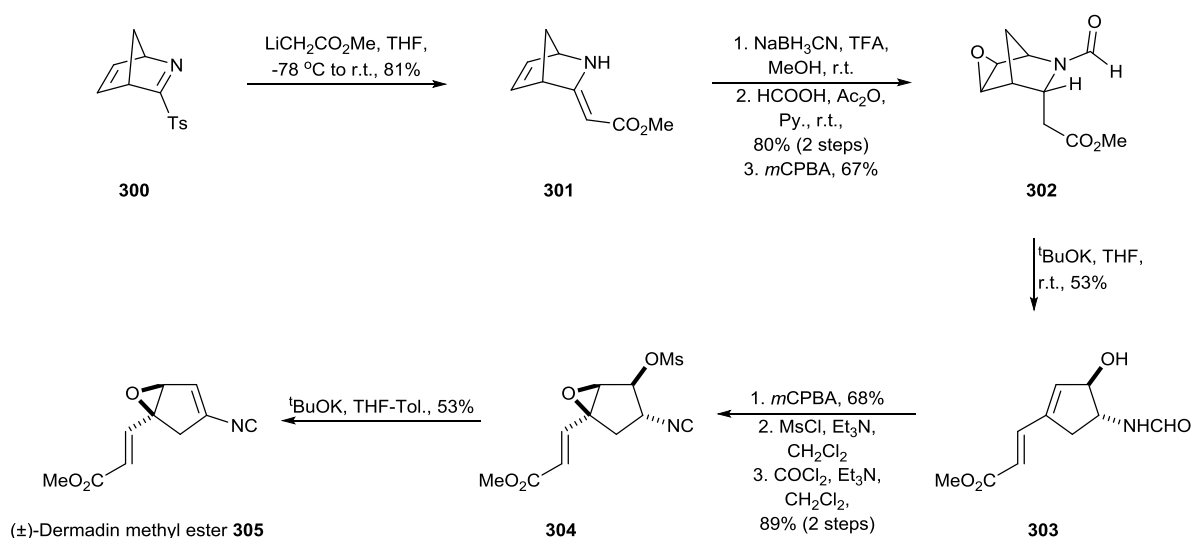


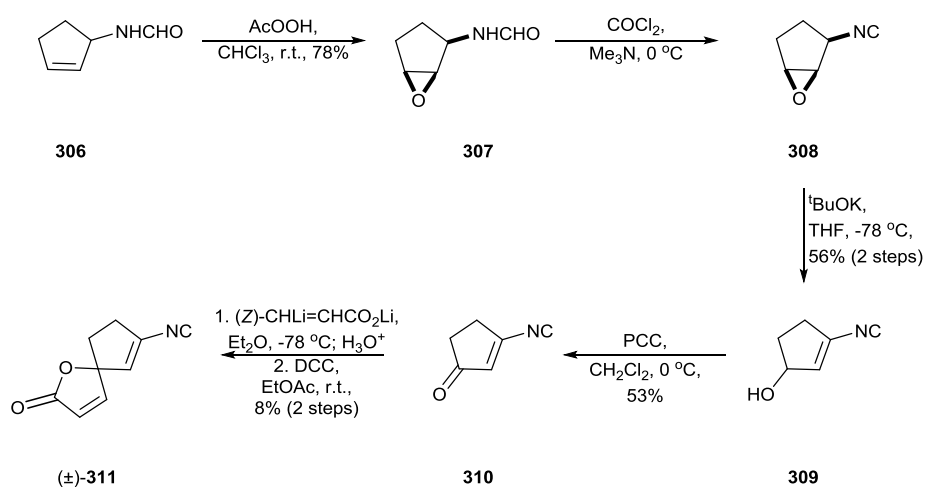
Figure 16 Biological activities of cyclopentyl isocyanide natural products **13**, **282**, **283** and **391**.<sup>117–120</sup>

The first synthesis of this family of natural products was carried out by Fukuyama and Yung (Scheme 31),<sup>115</sup> who synthesised (±)-methyl 3-(3-isocyano-6-oxabicyclo[3.1.0]hex-2-en-5-yl)-2-propenoate **305** as a stable analogue of its parent acid dermadin **282**. Their synthesis started with treatment of *C*-tosyl-aza-diene **300** with the lithium enolate of methyl acetate to afford  $\alpha,\beta$ -unsaturated ester **301**. Conjugate reduction of ester **301** with sodium cyanoborohydride, followed by *N*-formylation with formic acid/acetic anhydride in the presence of pyridine and epoxidation of its alkene functionality with *m*CPBA generated *exo*-epoxide **302**. Potassium *tert*-butoxide was then used as a base to fragment its bicyclic system, with concomitant elimination of the epoxide group occurring to afford diene-ester **303**. Subsequent *syn*-mono-epoxidation of diene ester **303** with *m*CPBA, was followed by *O*-mesylation and dehydration of the formamide functionality ( $\text{COCl}_2/\text{Et}_3\text{N}$ ) to afford vinyl-isocyanide **304** in 89% yield. Dermadin methyl ester **305** was then produced in 53% yield *via* treatment of ester **304** with potassium *tert*-butoxide, resulting in E1cB elimination of the mesylate group to introduce its vinyl group.<sup>115</sup>



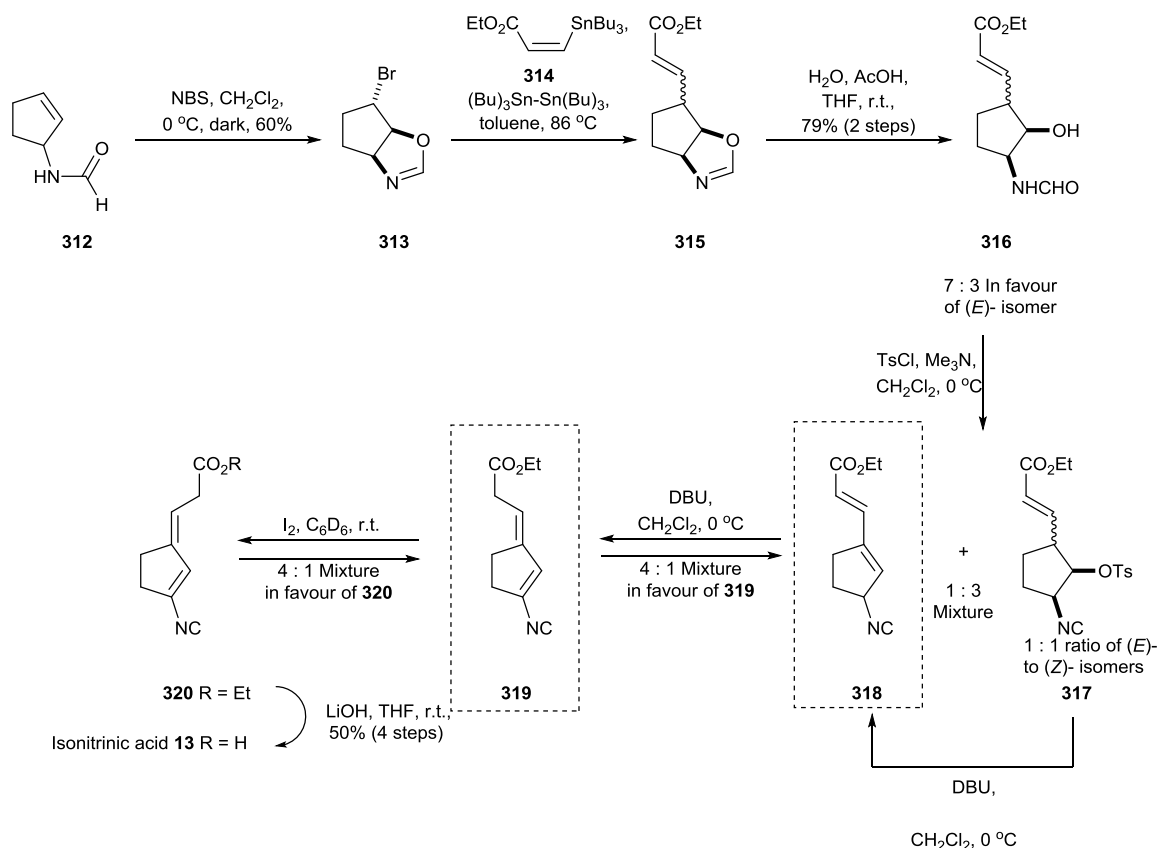
Scheme 31 Fukuyama's synthesis of dermadin methyl ester (±)-305.<sup>115</sup>

Baldwin *et al.* employed phosgene as an alternative formamide dehydrating agent in their low-yielding synthesis of the spirocyclic cyclopentyl isocyanide (±)-311 (Scheme 32),<sup>121</sup> which commenced with treatment of cyclopentene 306 with peracetic acid to generate *syn*-epoxide 307 that was then dehydrated with  $\text{COCl}_2$  to afford isocyanide 308. This isocyanide 308 underwent base promoted elimination of its epoxy group to afford vinyl-isocyanide 309 followed by oxidation of its allylic alcohol group with PCC to afford enone 310. The synthesis of the natural product 311 was completed *via* addition of (*Z*)-β-lithioacrylate to γ-ketoisocyanide 310, followed by ring closure using *N,N'*-dicyclohexylcarbodiimide (DCC) to facilitate lactonisation.<sup>121</sup>

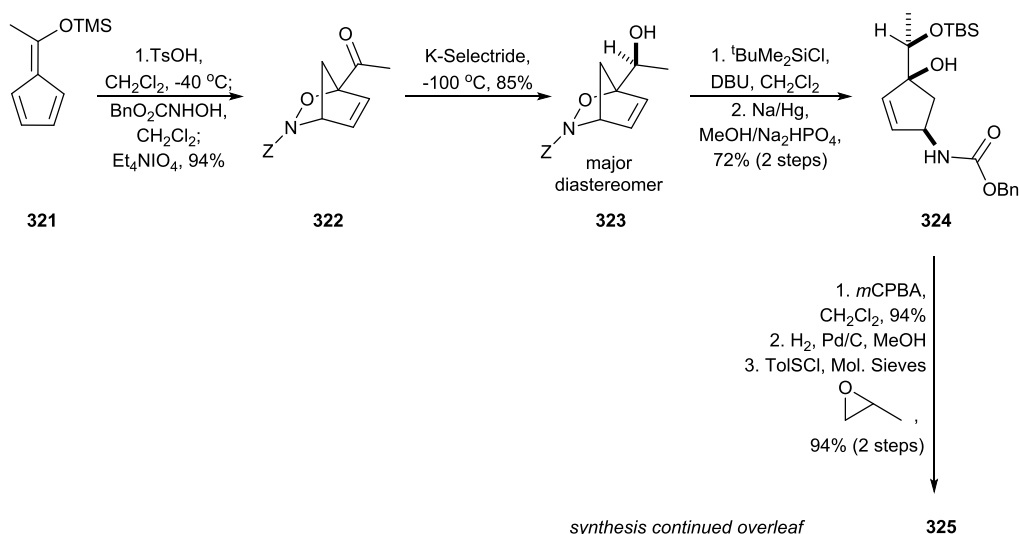


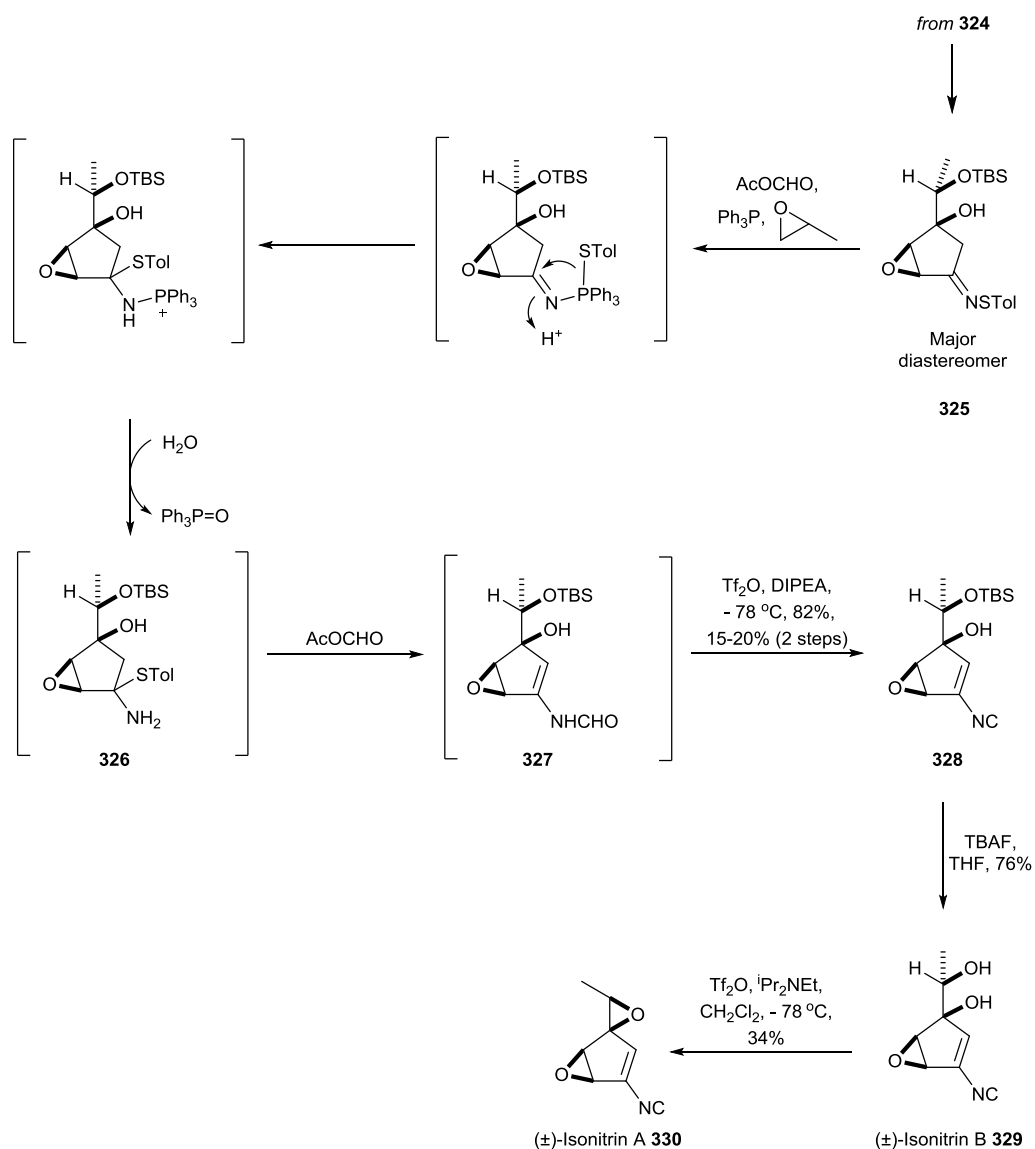
Scheme 32 Baldwin's synthesis of spirocyclic dienyl isocyanide (±)-311.<sup>121</sup>

Baldwin and co-workers carried out the synthesis of another cyclopentanoid isocyanide antibiotic isonitrinic acid F **13** produced by the fungi genus *Trichoderma* (Scheme 33).<sup>122</sup> Formamide **312** was treated with *N*-bromosuccinimide (NBS) in the dark to afford bicyclic dihydrooxazole **313** that underwent Stille coupling with a  $\beta$ -stannyl acrylate **314** to afford  $\alpha,\beta$ -unsaturated ester **315**. Mild hydrolysis of the oxazole fragment of **316** using aqueous acetic acid was followed by treatment of the resultant formamides **316** (7 : 3 mixture in favour of (*E*)-isomer) with TsCl/Et<sub>3</sub>N which gave a 3 : 1 mixture of *O*-tosyl-isocyanide **317** (50 : 50 mixture of (*E*)-/(*Z*)- isomers) and dienyl isocyanide **318**. Treatment of this mixture of isocyanides with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in elimination/alkene isomerisation to afford a 4 : 1 mixture of diene **319** and its regioisomer **318** under thermodynamic control. Treatment of diene **319** with iodine in d<sup>6</sup>-benzene resulted in a 4 : 1 mixture of dienes **320** and **319** that could be separated by chromatography. Finally, subsequent hydrolysis of diene ester **320** with LiOH in THF gave isonitrinic acid F **13**.<sup>122</sup>


 Scheme 33 Baldwin's synthesis of isonitrinic acid F **13**.<sup>122</sup>

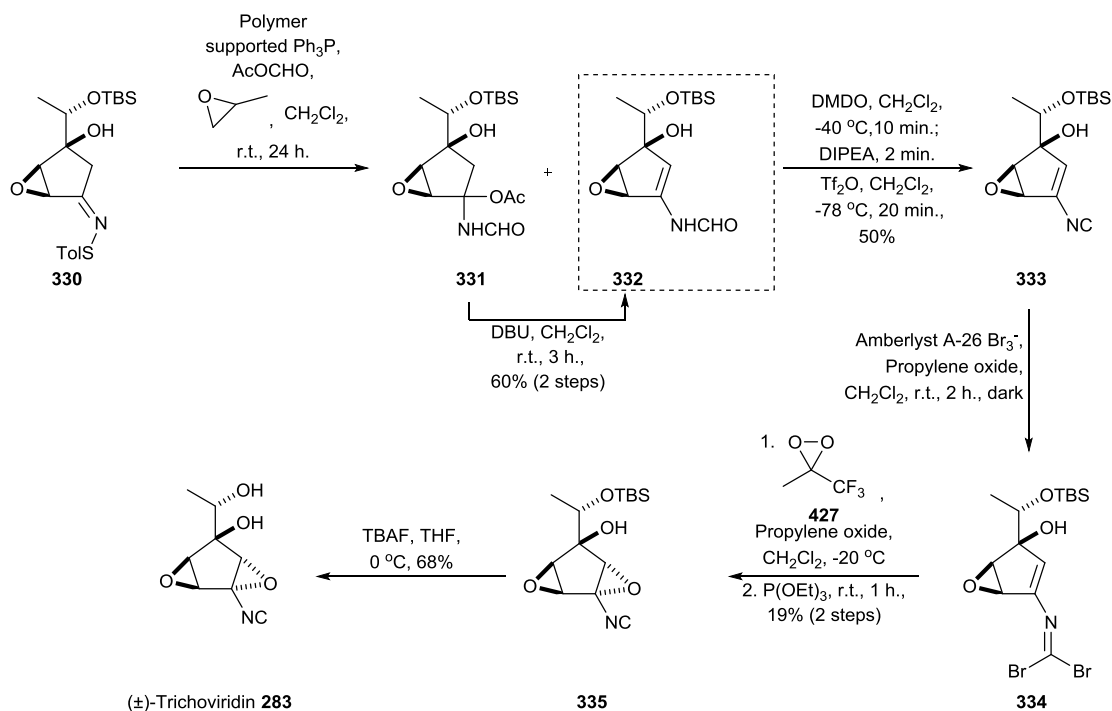
Baldwin also reported the total synthesis of isonitrin B **329** (Scheme 34),<sup>123</sup> another fungal cyclopentyl natural product isolated from the genus *Trichoderma*. *O*-Silyl protected fulvene **321** was treated with *p*TSA to afford 1-acetyl cyclopentadiene that then underwent a Diels-Alder reaction with a nitroso dienophile generated *in situ* from the action of tetraethylammonium periodate on *N*-hydroxycarbamate. The resultant bicyclic ketone **322** was selectively reduced *via* treatment with K-Selectride to afford an alcohol **323** that was then *O*-silyl protected. Subsequent treatment of the resultant bicyclic silyl ether with sodium amalgam in buffered methanol resulted in cleavage of its *N*-*O* bond to afford cyclopentenyl carbamate **324** that underwent a directed epoxidation reaction with *m*CPBA to afford a *syn*-epoxide. The Cbz-group of this epoxide was then removed *via* hydrogenolysis to afford an amine intermediate that was reacted with tolylthiooxime and TsCl in the presence of propylene oxide and molecular sieves to afford tolylthiooxime **325**. This tolylthiooxime **325** was then treated with triphenylphosphine and acetic formic anhydride in the presence of propylene oxide, which resulted in insertion of triphenylphosphine into the *N*-STol bond, followed by rearrangement and loss of triphenylphosphine oxide, to afford an amine **326** that was then *N*-formylated with formic acetic anhydride to give formamide **327** in low yield. Dehydration of formamide **327** was achieved *via* treatment with triflic anhydride and DIPEA to afford vinyl isocyanide **328**, with isonitrin B **329** then being generated by desilylation of its hydroxyl groups using TBAF.<sup>123</sup> Baldwin subsequently reported the use of triflic anhydride and DIPEA as a simple way of converting the vicinal diol fragment of isonitrin B **329** into the second epoxide functionality of isonitrin A **330**.<sup>124</sup>




 Scheme 34 Baldwin's synthesis of (±)-isonitrin A **330** and (±)-isonitrin B **329**.<sup>123, 124</sup>

Baldwin *et al.* have also described the use of dimethyldioxirane (DMDO) as a dehydrating agent for the conversion of an *N*-formamide group into an isocyanide group as part of their 1996 synthesis of (±)-trichoviridin **283** (Scheme 35),<sup>125</sup> which is known to inhibit mushroom tyrosinase and melanin formation in larval haemolymph.<sup>126</sup> Treatment of tolylthiooxime **330** with polymer bound  $\text{Ph}_3\text{P}$  and acetic formic anhydride in the presence of propylene oxide as an acid scavenger generated a mixture of  $\alpha$ -acetoxy-formamide **321** and vinyl-formamide **332**.  $\alpha$ -Acetoxy formamide **331** was eliminated to give its vinylogous equivalent **332** via treatment with DBU, which was then dehydrated *in situ* via sequential treatment with DMDO and triflic anhydride to afford vinyl-isocyanide **333** as the only isolable product in 50% yield. Isocyanide **333** was then protected as its dibromoimino derivative **334** via treatment with amberlyst A-26

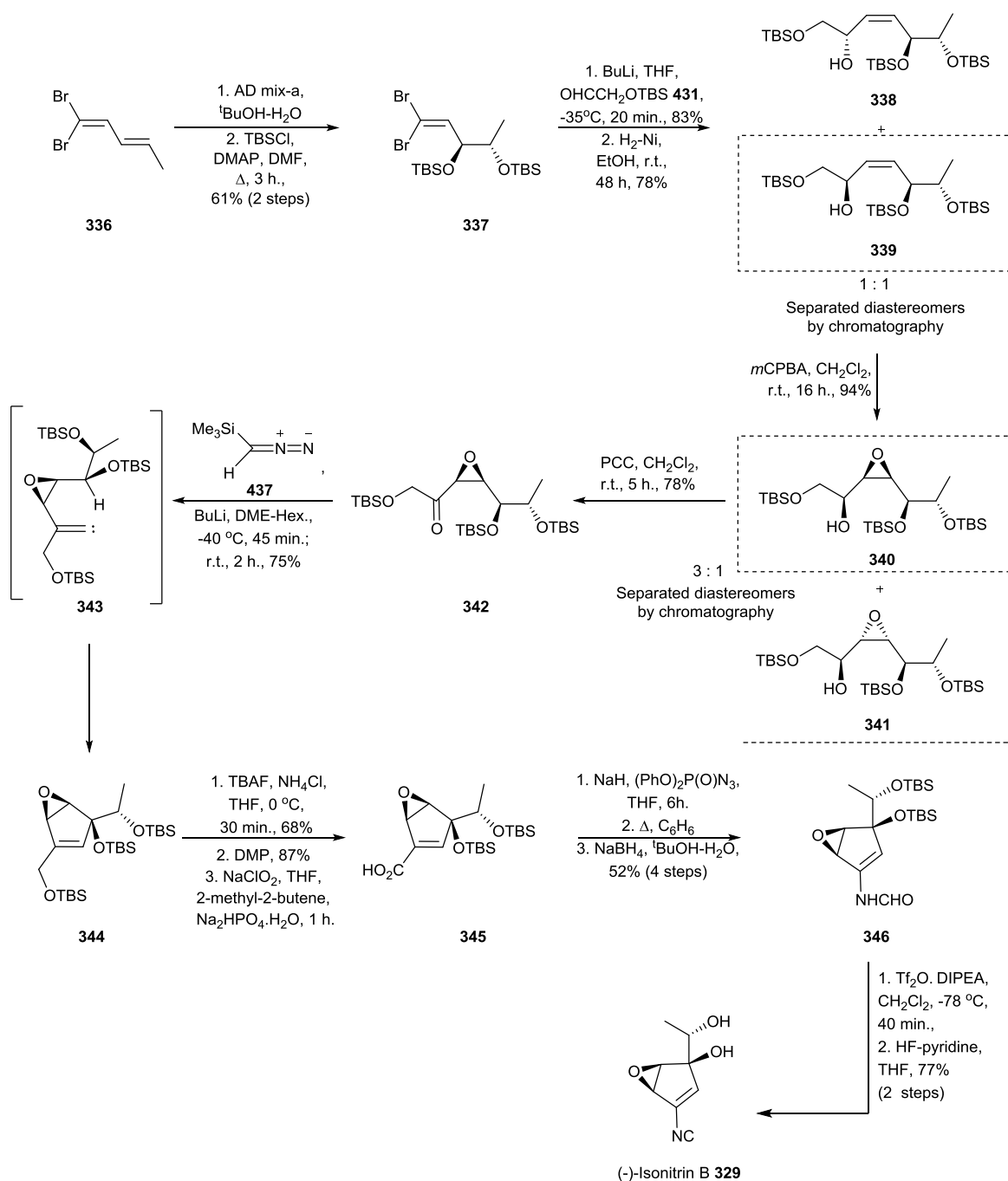
$\text{Br}_3^-$ , allowing for methyl(trifluoromethyl)dioxirane to be used to introduce the second *anti*-epoxide ring of a *bis*-epoxide intermediate. Deprotection of the dibromoimino functionality of this *bis*-epoxide was then achieved *via* treatment with triethylphosphite to afford isocyanide **335** that was *O*-silyl deprotected with TBAF to give ( $\pm$ )-trichoviridin **283**.<sup>125</sup>



Scheme 35 Baldwin's synthesis of ( $\pm$ )-trichoviridin **283**.<sup>125</sup>

Taber *et al.* subsequently reported an enantioselective synthesis of (-)-isonitrin B **329** (Scheme 36),<sup>127</sup> using chiral allylic alcohol **340** that was prepared in four steps from dibromo-diene **336** involving: (i) Sharpless asymmetric dihydroxylation reaction; (ii) *bis*-*O*-TBS protection to afford **337**; (iii) lithiation of the dibromoalkene fragment to afford an alkynyl anion that underwent a non-selective aldol reaction with aldehyde **338**; (iv) partial hydrogenation of the resultant alkyne to afford a *cis*-alkene. The resultant mixture of allylic diols **339/340** were separated by chromatography and alkene **340** subjected to a directed epoxidation reaction using *m*CPBA to afford a 3 : 1 mixture of epoxides **341** and **342** that were separated by chromatography.<sup>127</sup> Epoxide **341** was oxidised with PCC to afford a ketone **343** that was treated with the anion of (trimethylsilyl)diazomethane **344** (TMSDM) to afford an alkylidene carbene species **345** that underwent an intramolecular C-H insertion reaction to give cyclopentenyl epoxide **346**. The primary silyloxy group of epoxide **346** was deprotected *via* treatment with TBAF and the resultant alcohol oxidised in a stepwise fashion to afford its corresponding acid **347** that was

then treated with DPPA to give its corresponding acyl azide. Thermolysis of this acyl azide resulted in Curtius rearrangement to afford an isocyanate intermediate that was reduced with NaBH<sub>4</sub> to afford the desired formamide **348**. Triflic anhydride in the presence of DIPEA was then used to facilitate dehydration of formamide **348** into its corresponding isocyanide, which upon HF/pyridine mediated *O*-silyl deprotection generated (-)-isonitrin B **329**.<sup>127</sup>

Scheme 36 Taber's synthesis of (-)-isonitrin B **329**.<sup>127</sup>

### 1.1.7. Syntheses of monoterpene indole alkaloid isocyanides

More than 30 different chlorinated and non-chlorinated monoterpene indole isocyanides have been isolated from filamentous blue-green algae, where they have often been isolated in the presence of structurally related isothiocyanate analogues. This class of isocyanide natural product include the hapalindoles, fischerindoles, welwitindolinones and ambiguines, all of which contain an isocyanide group attached to C<sub>11</sub> of their respective polycyclic skeletons. A number of these naturally occurring isocyanides have been shown to exhibit useful biological activity (Figure 17), including antibacterial, antimycotic, antifungal, antialgal and insecticidal activity. The hapalindoles, isolated from the blue-green algae *Hapalosiphon fontinalis* (Ag.) Bornet (strigonemataceae), are indole alkaloids that Moore *et al.* originally isolated through culturing a terrestrial cyanophyte present in a soil sample.<sup>128,129</sup> Moore and co-workers were also responsible for isolating the first ambiguines, fischerindoles and welwitindolinones from cultures of *Hapalosiphon welwitshii* and *Westiella intricata*.<sup>130,131</sup>

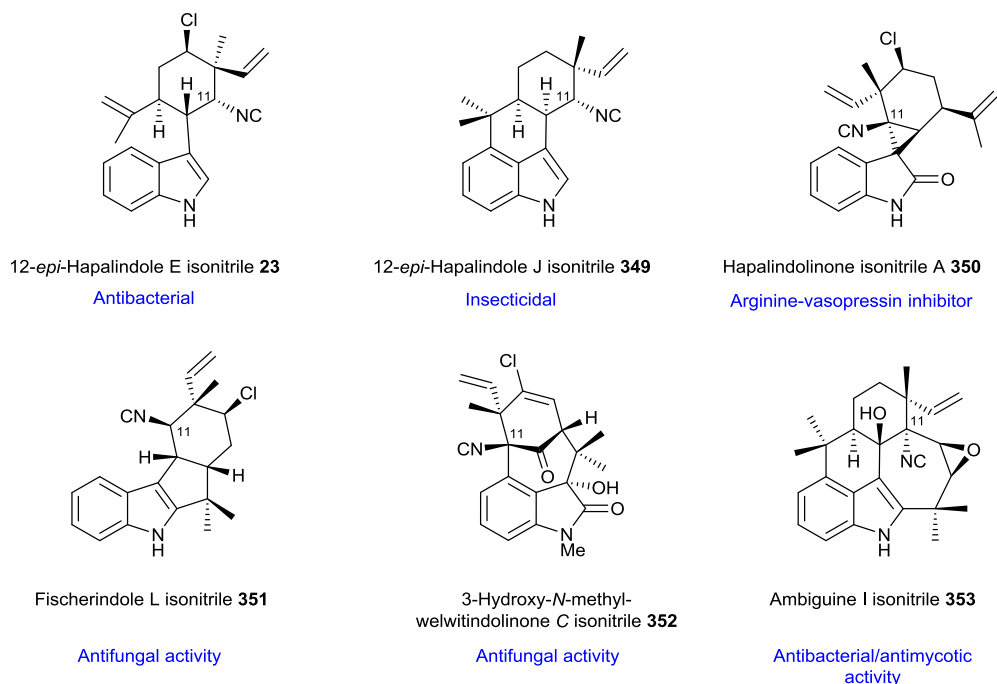
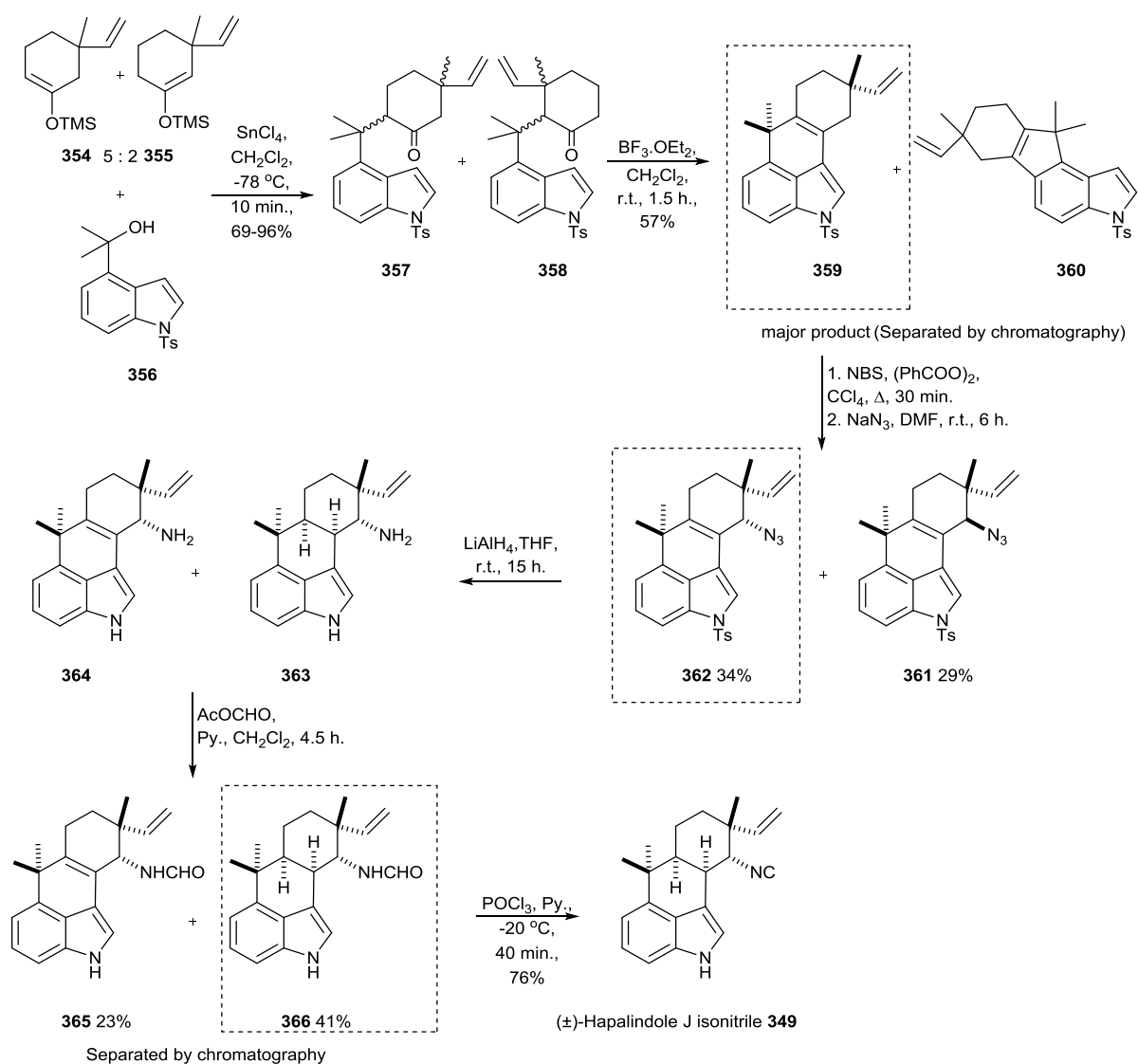


Figure 17 Monoterpene indole alkaloid isocyanides **23**, **349-353**.<sup>130,132-136</sup>

The first synthesis of hapalindole J isonitrile **349** was described by Natsume and co-workers in 1989 (Scheme 37).<sup>137,138</sup> Their synthesis commenced with SnCl<sub>4</sub> catalysed coupling of a 5 : 2 mixture of sily-enol ethers **354/355** with a tertiary cationic species derived from alcohol **356**, with the resultant mixture of structural isomers **357** and **358** then being treated with BF<sub>3</sub>·OEt<sub>3</sub>

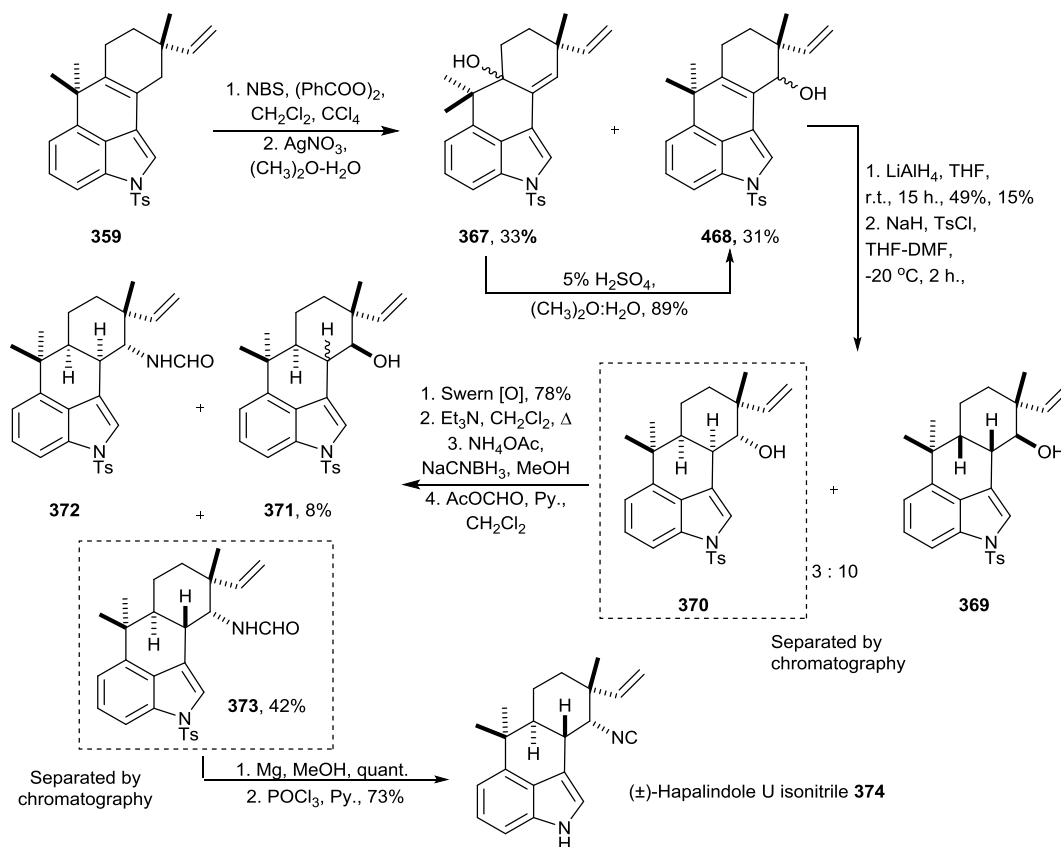


to induce electrophilic cyclisation of the indole ring onto its keto group to afford alkene-indole **359** and the unwanted structural isomer **360**. This alkene-indole **359** was then subjected to a free radical allylic bromination reaction using NBS and benzoyl peroxide, before being treated with sodium azide to afford an approximately 1 : 1 mixture of epimeric azides **361** and **362**. Azide **362** was then reduced with  $\text{LiAlH}_4$ , accompanied by *N*-detosylation of its indole fragment and partial reduction of its alkene bond to afford a mixture of amines **363** and **364** that were then treated with acetic formic anhydride to afford an approximate 1 : 2 mixture of the unsaturated *N*-formamide **365** and saturated *N*-formamide **366**. Formamide **366** was subsequently dehydrated to afford hapalindole J isonitrile **349** using  $\text{POCl}_3$  in pyridine.<sup>137,138</sup>

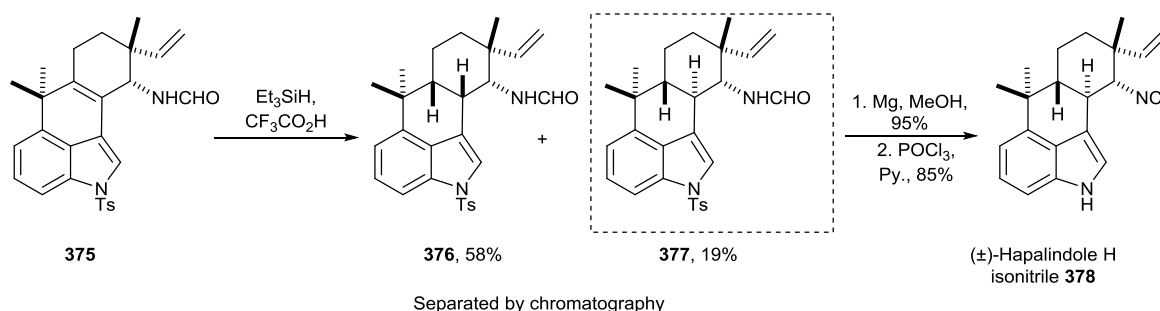


Scheme 37 Natsume's syntheses of (±)-hapalindole J isonitrile **349**.<sup>137,138</sup>

Natsume also reported the synthesis of hapalindoles U isonitrile **374** from an intermediate **359** previously used in his synthesis of hapalindole J isonitrile **349** (Scheme 38).<sup>139</sup> A 1 : 1 mixture of allylic alcohols **367** and **368** was generated *via* free radical allylic bromination of **359** with NBS followed by allylic hydroxylation using AgNO<sub>3</sub> in aqueous acetone. Separation and treatment of regioisomer **367** with H<sub>2</sub>SO<sub>4</sub> in the presence of aqueous acetone resulted in its conversion into allylic alcohol **368**. The alkene bond of **368** was then reduced with LiAlH<sub>4</sub>, which also resulted in unwanted *N*-detosylation, therefore the *N*-tosyl group was reintroduced *via* treatment of the crude reaction product with TsCl and NaH. The resulting 10 : 3 mixture of *N*-tosyl alcohols **369** and **370** was separated and the minor *cis*-fused epimer **370** subjected to Swern oxidation, followed by epimerisation with Et<sub>3</sub>N, Reductive animation of the resultant mixture of ketones with NH<sub>4</sub>OAc/NaCNBH<sub>3</sub>, followed by *N*-formylation with acetic formic anhydride in pyridine, resulted in a mixture of epimeric formamides **372** and **373**, as well as a small amount of the unwanted epimeric alcohols **371**. This complex mixture was separated to afford the desired *N*-tosyl formamide **373**, whose *N*-tosyl group was removed *via* treatment with magnesium in methanol, followed by formamide dehydration using POCl<sub>3</sub> in pyridine to afford (±)-hapalindole U isonitrile **374**.<sup>139</sup>

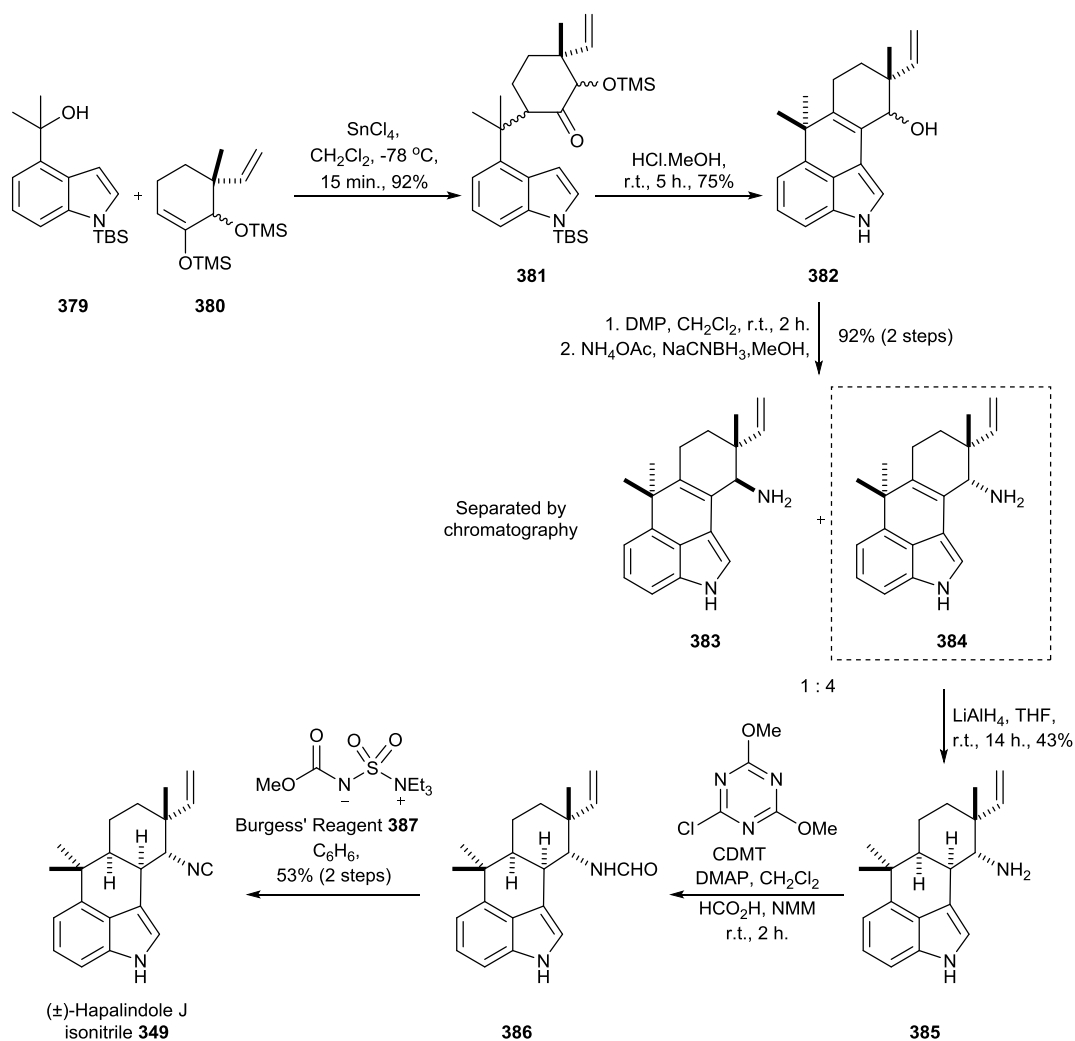
Scheme 38 Natsume's synthesis of (±)-hapalindole U isonitrile **374**.<sup>139</sup>

Natsume and co-workers also demonstrated that treatment of *N*-tosyl-formamide **375**<sup>137–139</sup> with Et<sub>3</sub>SiH in TFA resulted in reduction of its alkene bond to afford a mixture of reduced formamides **376** and **377** that could be separated *via* chromatography. The minor formamide **377** was then converted into (±)-hapalindole H isonitrile **378** using the same *N*-detosylation/formamide dehydration conditions described for (±)-hapalindole U isonitrile **378** (Scheme 39).<sup>139</sup>

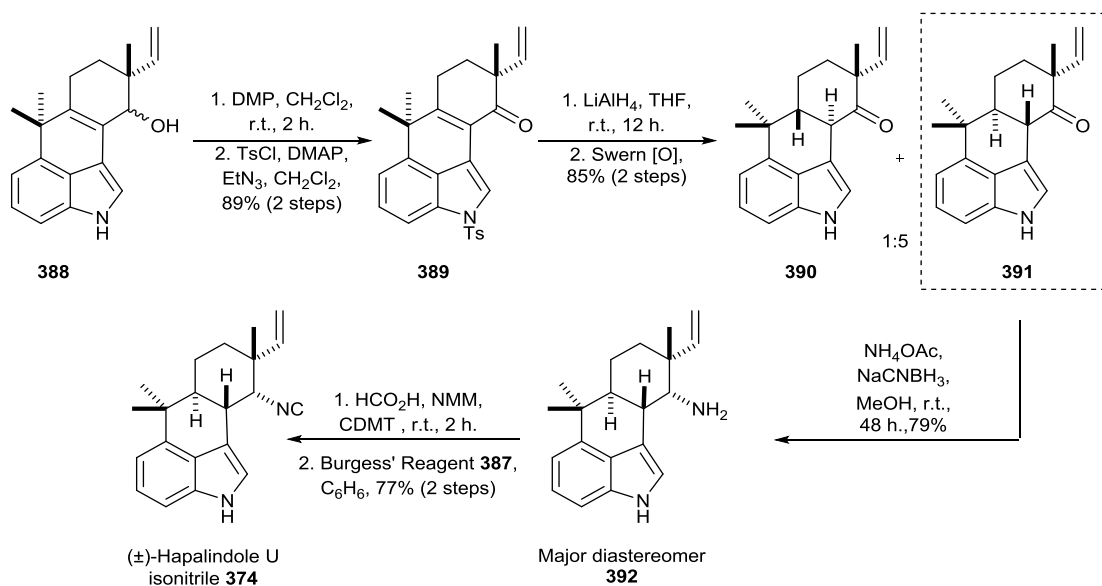


Scheme 39 Natsume's synthesis of (±)-hapalindole H isonitrile **378**.<sup>139</sup>

In 2012 Rafferty and Williams employed a similar approach for their synthesis of (±)-hapalindole J isonitrile **349** (Scheme 40) *via* a route that also involved SnCl<sub>4</sub> mediated coupling of a silyl enol ether **380** with a cationic species derived from the tertiary alcohol fragment of *N*-silyl-indole **379** to give indole **381** as a mixture of diastereomers.<sup>140</sup> Indole **381** was then cyclised *via* treatment with methanolic HCl with subsequent *O*-silyl-deprotection to generate the tetracyclic core of indole **382**, which was oxidised into a ketone intermediate that underwent reductive amination to afford a 1 : 4 epimeric mixture of amines **383** and **384**. After purification by chromatography, the *cis*-fused ring system of indole **385** was established *via* reduction of the cyclohexenyl bond of indole **385** with LiAlH<sub>4</sub>, with the amino substituent of indole **384** then being *N*-formylated *via* treatment with formic acid, 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), a catalytic amount of *N,N*-dimethylaminopyridine (DMAP) and *N*-methylmorpholine (NMM) in CH<sub>2</sub>Cl<sub>2</sub>. The isocyanide functionality of (±)-hapalindole J isonitrile **349** was then introduced by dehydrating the formamide functionality of indole **386** using Burgess' reagent **387** in benzene.<sup>140</sup>


 Scheme 40 Williams' synthesis of (±)-hapalindole J isonitrile **349**.<sup>140</sup>

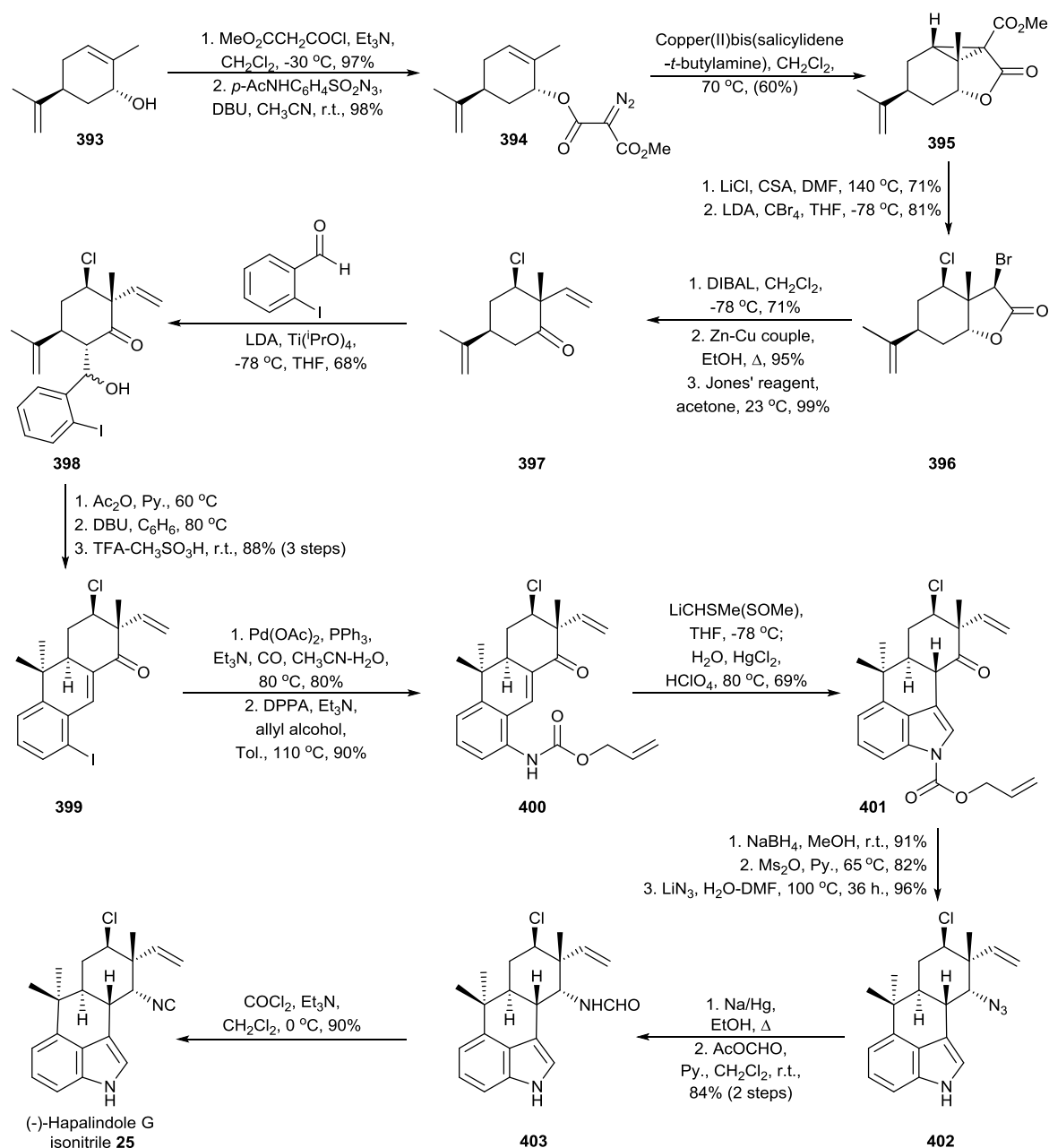
The same paper also reported the synthesis of the *trans*-fused ring system of (±)-hapalindole U isonitrile **374** (Scheme 41) from the epimeric alcohol intermediate **474** that was oxidised using DMP to afford a ketone, whose indole nitrogen was *N*-tosylated to afford indole **389**. The alkene bond of indole **389** was then reduced with  $\text{LiAlH}_4$ , with concomitant *N*-detosylation, to produce an alcohol (mixture of epimers) that was oxidised under Swern conditions to afford *trans*-fused ketones **390** and **391**. The major diastereomer **391** was purified by chromatography and then transformed into (±)-hapalindole U isonitrile **374** (*via* amine **492**) using the same sequence of synthetic steps used for completion of the synthesis of (±)-hapalindole U isonitrile **374**.<sup>140</sup>



Scheme 41 Williams' synthesis of (±)-hapalindole U isonitrile **374**.<sup>140</sup>

Fukuyama and Chen reported the synthesis of (-)-hapalindole G isonitrile **25**, which is an antibacterial, antimycotic and antialgal isocyanide that was isolated from terrestrial alga *Hapalosiphon fontinalis* (scheme 42).<sup>141</sup> (-)-Trans-carveol **393** was esterified with methyl(chloroformyl)acetate, followed by diazo transfer using para-AcNHC<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>N<sub>3</sub> to afford a diazomalonate **394** that underwent a copper catalysed intramolecular cyclopropanation reaction to afford tricyclic cyclopropyl ester **395**. Heating cyclopropyl ester **395** with lithium chloride in the presence of camphor sulfonic acid (CSA) resulted in stereoselective ring opening of the cyclopropane ring by chloride anion, accompanied by decarboxymethylation to afford a lactone whose enolate was brominated (LDA, CBr<sub>4</sub>) to afford bromolactone **396**. Bromolactone was reduced with DIBAL to afford a diol whose bromohydrin fragment underwent a Boord-like elimination reaction on treatment with zinc-copper couple to afford an alkene intermediate whose secondary alcohol group was then oxidised with Jones' reagent to afford chloroketone **397**. This chloroketone **397** then underwent a titanium mediated aldol reaction with 2-iodobenzaldehyde to give an epimeric mixture of hydroxy-ketones **398**. This mixture of epimeric alcohols **398** were converted into their corresponding acetates that underwent DBU facilitated elimination reactions to afford an enone that was treated with TFA to initiate a Friedel-Crafts like cyclisation reaction that gave tricyclic iodo-ketone **399**. The indole component was then constructed by a palladium facilitated carbonylation reaction to afford an aryl carboxylic acid intermediate that was treated with DPPA to afford an acyl azide intermediate that underwent a Curtius rearrangement with allyl alcohol to afford allyl-carbamate **400**. The Enone of **400** then underwent a conjugate addition reaction with

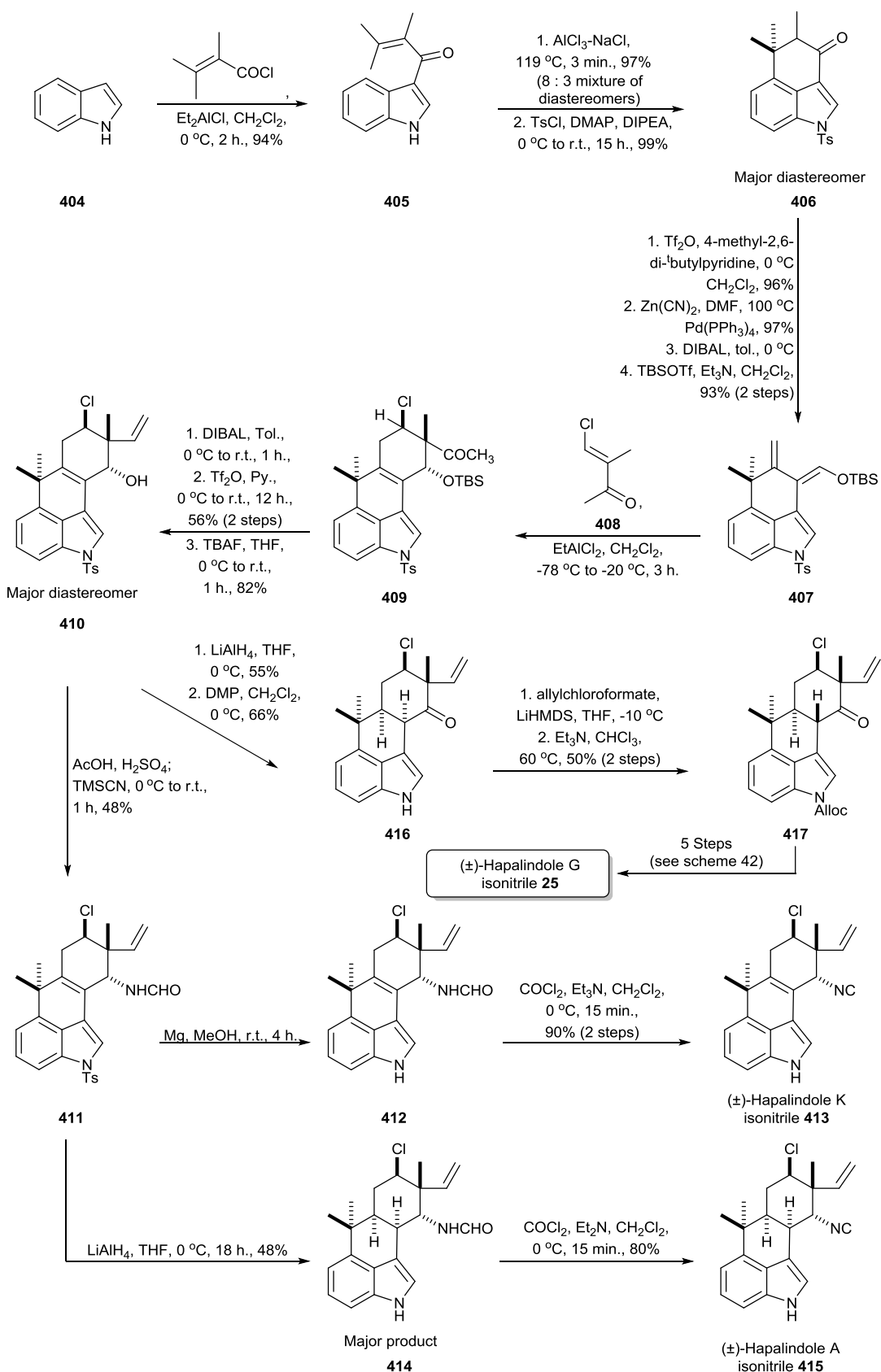
LiCHSMe(SOMe), followed by hydrolysis with  $\text{HgCl}_2$ /perchloric acid to afford an aldehyde intermediate that condensed with the nitrogen atom of the carbamate group to afford the C1-homologated N-alloc-indole **401**. Stereoselective reduction of the keto group of indole **401** gave a  $\beta$ -alcohol that was *O*-mesylated and treated with lithium azide to afford  $\alpha$ -azide **402**. Reduction of azide **402** with sodium/mercury amalgam and subsequent formylation produced **403** that was then dehydrated using  $\text{COCl}_2$  and  $\text{Et}_3\text{N}$  to afford hapalindole G isonitrile **25**.<sup>141</sup>



Scheme 42 Fukuyama's synthesis of (-)-hapalindole G isonitrile **25**.<sup>141</sup>

Hapalindole A isonitrile **415** and Hapalindole K isonitrile **413** have been shown to inhibit green algae photosynthesis, with Hapalindole A isonitrile **415** being found to demonstrate both antialgal and antimycotic activity towards *H. fontinalis*.<sup>131</sup> For their racemic syntheses (Scheme 43),<sup>142</sup> Johnston and Chandra carried out a Friedel-Crafts acylation reaction of indole **404** with  $\alpha$ -methyl tiglic acid to afford ketone **405** whose alkene fragment was cyclised onto its aryl ring *via* heating in molten  $\text{AlCl}_3\text{-NaCl}$  to generate a tricyclic ketone that was *N*-tosylated to afford indole **406**. Ketone **406** was then converted into its corresponding enol triflate that was treated with  $\text{Zn}(\text{CN})_2$  and  $\text{Pd}(\text{PPh}_3)_4$  to give an  $\alpha,\beta$ -unsaturated nitrile that was reduced with DIBAL to afford an enal that was converted into its corresponding silyl enol ether **407** *via* treatment with TBSOTf and  $\text{Et}_3\text{N}$ . Tetracyclic *N*-tosyl-indole **409** was then generated as a major diastereomer from Lewis acid mediated intermolecular Diels-Alder reaction of diene **407** with  $\beta$ -chloro-enone **408** in the presence of  $\text{EtAlCl}_2$ . Reduction of ketone **409** with DIBAL was followed by treatment with triflic anhydride/pyridine resulting in elimination of the resultant triflate to afford an alkene intermediate that was *O*-silyl deprotected using TBAF to afford alcohol **410**. A Ritter reaction involving treatment of the acetate of alcohol **410** with  $\text{TMSCN}$  was then used to generate formamide **411** as a precursor for the synthesis of both ( $\pm$ )-hapalindole K isonitrile **413** and hapalindole A isonitrile **415**. For ( $\pm$ )-hapalindole K isonitrile **413**, magnesium methoxide was used to effectively *N*-detosylate the indole ring of formamide **411**, with the formamide functionality of indole **412** then being dehydrated by the action of  $\text{COCl}_2/\text{Et}_3\text{N}$  in 90% yield.<sup>142</sup> For ( $\pm$ )-hapalindole A isonitrile **415**,  $\text{LiAlH}_4$  was used to reduce the cyclohexenyl functionality to afford a *cis*-fused ring and facilitate *N*-detosylation to afford indole **414**, with dehydration of the resultant formamide with  $\text{COCl}_2/\text{Et}_3\text{N}$  once again affording its isocyanide functionality.<sup>142</sup> The authors also claimed a formal synthesis of ( $\pm$ )-hapalindole G isonitrile **25**<sup>142</sup> involving reduction of the cyclohexenyl bond and *N*-detosylation of alcohol **410** with  $\text{LiAlH}_4$  to afford a *cis*-fused alcohol that was oxidised with DMP to afford ketone **416**. The nitrogen atom of indole **416** was protected as an *N*-allyl carbamate, followed by treatment with  $\text{Et}_3\text{N}$  which resulted in epimerisation to afford the *trans*-fused ring junction of ketone **417** that had previously been transformed into (-)-hapalindole G isonitrile **25** in five steps by Fukuyama and Chen.<sup>141</sup>

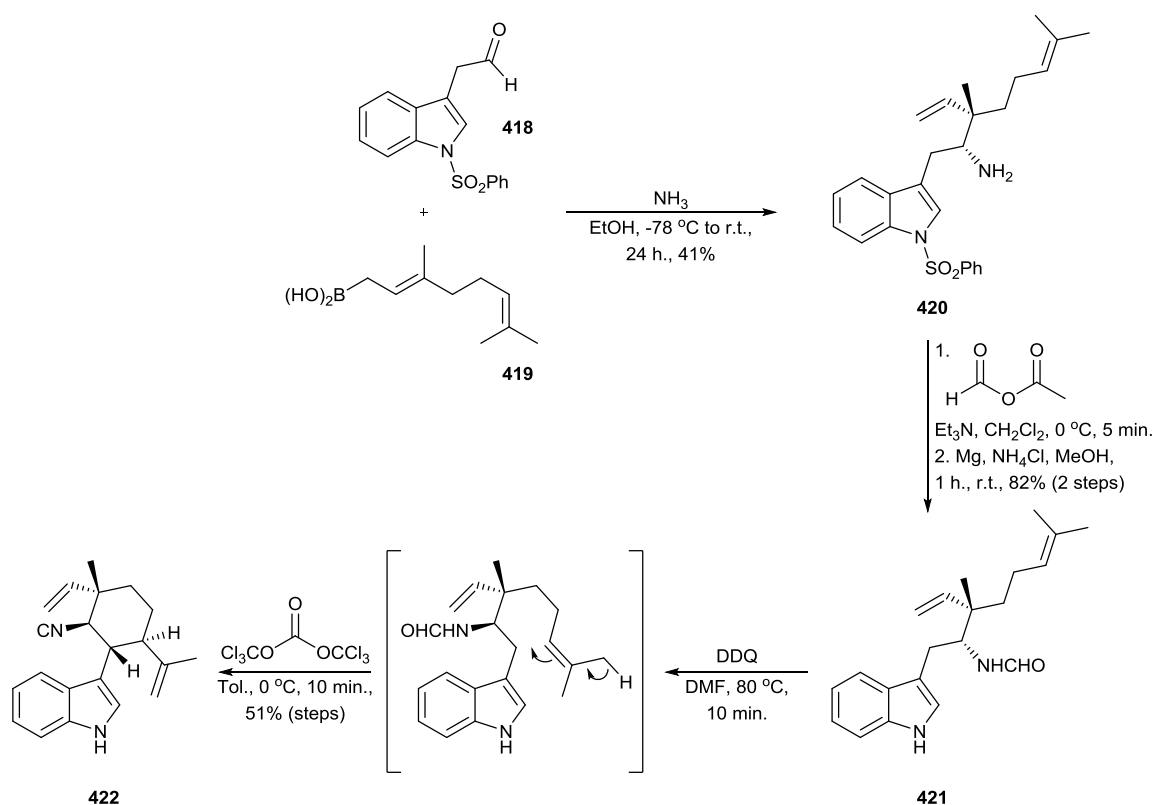
### Methodology for the synthesis of isocyanide containing natural products



Scheme 43 Johnston's syntheses of (±)-hapalindole G isonitrile **25**, (±)-hapalindole K isonitrile **503**, (±)-hapalindole A isonitrile **505**.<sup>142</sup>



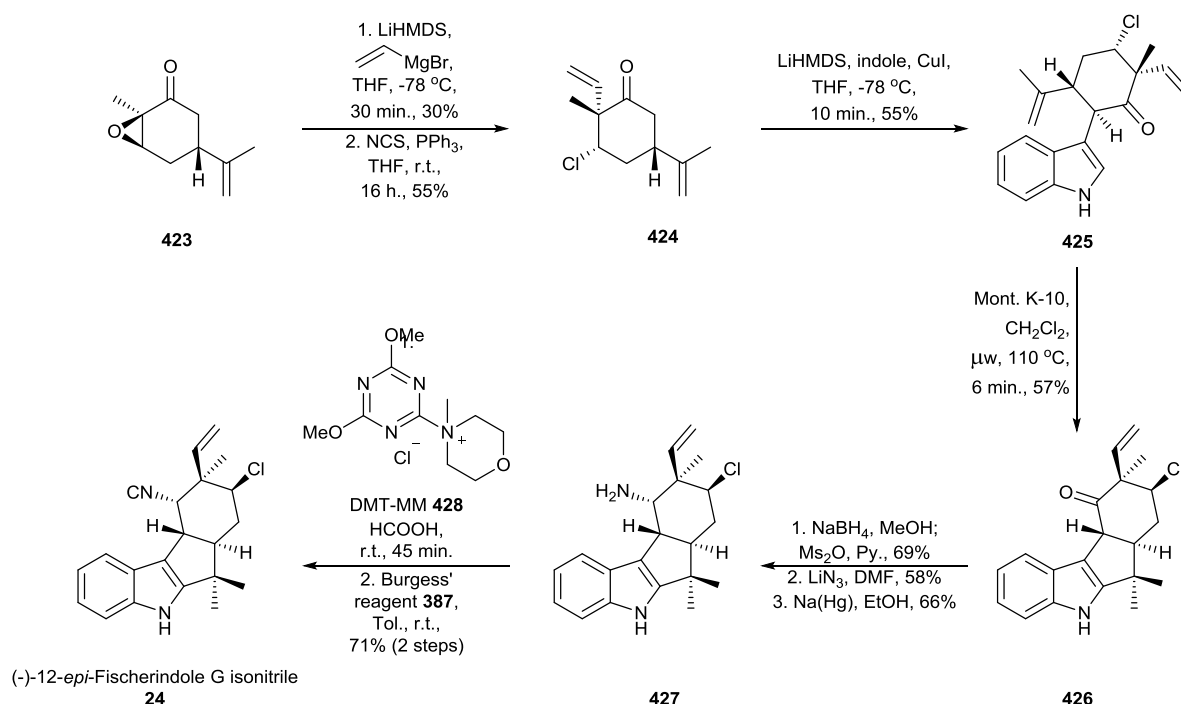
Li reported the synthesis of 12-*epi*-hapalindole Q isonitrile **422** via a bioinspired oxidative cyclisation strategy (Scheme 44).<sup>143</sup> Coupling of aldehyde **418** with *cis*-boronic acid **419** provided amine **420**, which was subsequently formylated using acetic formic anhydride and the indole portion of **420** de-sulfonated using solvated magnesium to afford diene **421**. Upon exposure to Sc(OTf)<sub>3</sub> and DDQ, diene **421** underwent oxidative cyclisation to provide a cyclic formamide that was dehydrated using triphosgene and triethylamine base, providing racemic 12-*epi*-hapalindole Q isonitrile **422**.<sup>143</sup>



Scheme 44 Li's synthesis 12-*epi*-hapalindole Q isonitrile **421**.<sup>143</sup>

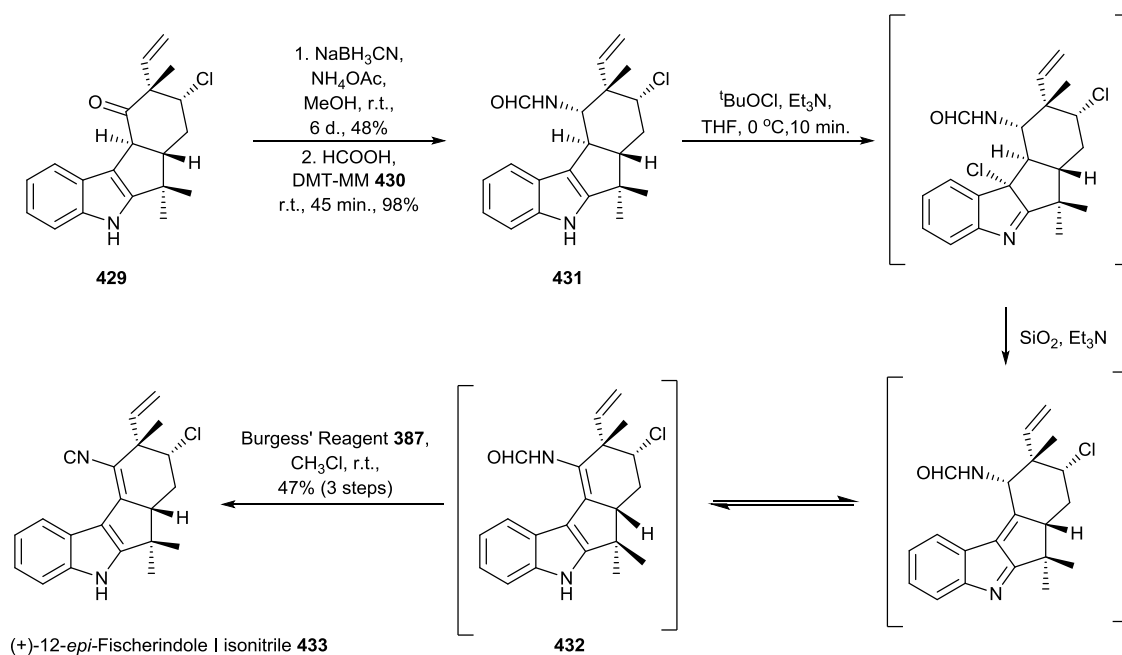
Baran and Richter have reported an elegant 'protecting group free' syntheses of *ent*-(-)-12-*epi*-fischerindole G isonitrile **24** (Scheme 45) and (+)-12-*epi*-fischerindole I isonitrile **433** (scheme 46).<sup>22,144</sup> Their synthesis of *ent*-12-*epi*-fischerindole G isonitrile **24** commenced with enolisation of (*R*)-carvone oxide **423**, with lithium bis(trimethylsilyl)amide (LiHMDS) followed by regioselective addition of vinyl magnesium bromide to the  $\alpha$ -position of its epoxide fragment. This gave an alcohol intermediate containing a quaternary stereocentre that was treated with *N*-chloro-succinimide (NCS)/ $\text{Ph}_3\text{P}$  to afford  $\beta$ -chloroketone **424** with inversion of configuration. Copper catalysed coupling of the lithium enolate of chloroketone **424** with indole then gave an

indole-ketone **425** whose isopropenyl fragment was cyclised onto to its aryl ring by heating with a clay catalyst (Montmorillonite K-10) in dichlorethane in a microwave to afford the tetracyclic core of indole **426**. Reduction of ketone **426** with NaBH<sub>4</sub> from its  $\alpha$ -face was followed by *O*-mesylation, displacement with LiN<sub>3</sub>, and reduction of the resulting azide using sodium-mercury amalgam to afford amine **427**. Amine **427** was then *N*-formylated with formic acid using the coupling agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) **428** and dehydrated using Burgess' reagent **387** to afford *ent*-(-)-*epi*-fischerindole G isonitrile **24**.<sup>22,144</sup>



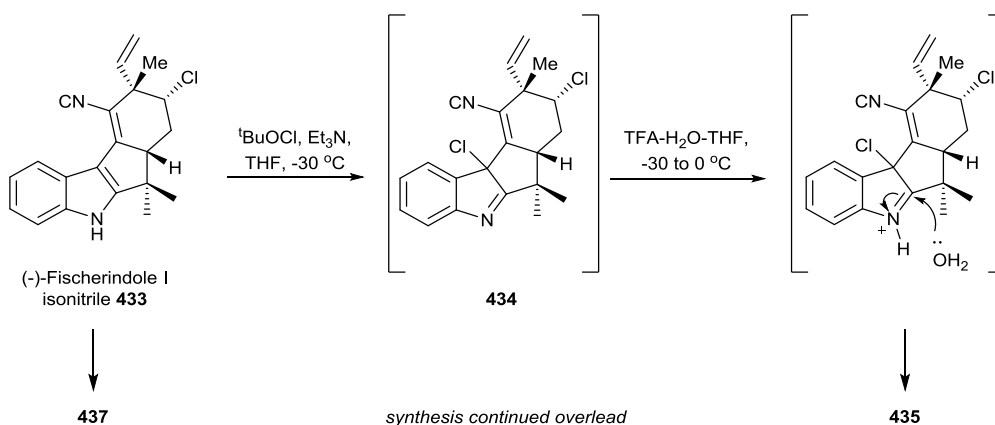
Scheme 45 Baran's synthesis of (-)-12-*epi*-fischerindole G isonitrile **24**.<sup>22,144</sup>

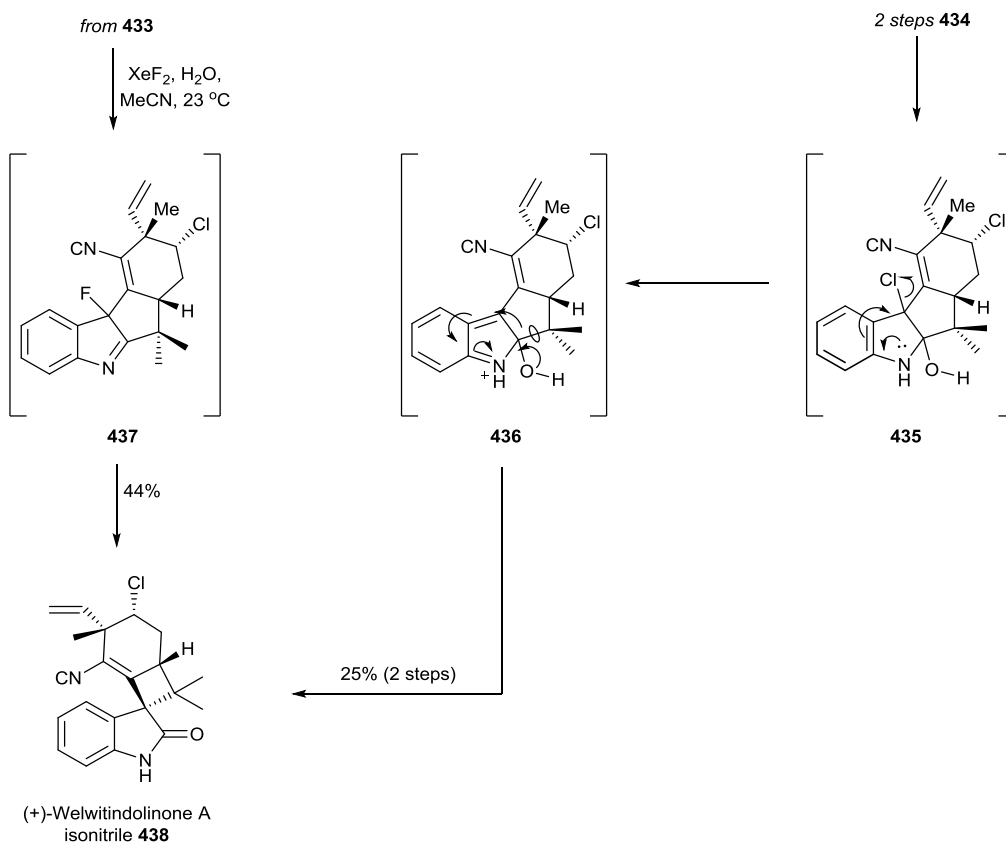
Alternatively, for the synthesis of (+)-12-*epi*-fischerindole I isonitrile **433**, ketone **429** was reductively aminated with NH<sub>4</sub>OAc/NaBH<sub>3</sub>CN to afford an amine that was *N*-formylated *via* treatment with formic acid and DMT-MM **430** (Scheme 46). Treatment of formamide **431** with the oxidant *tert*-butyl hyperchlorite and Et<sub>3</sub>N, followed by deactivated silica gel (Et<sub>3</sub>N), resulted in a cascade of chlorination/dehydrochlorination/tautomerisation reactions to give vinyl-formamide **432**, which was dehydrated *via* treatment with Burgess' reagent **387** to afford (+)-12-*epi*-fischerindole I isonitrile **433**.<sup>22,144</sup>



Scheme 46 Baran's synthesis of (+)-12-*epi*-fischerindole I isonitrile **433**.<sup>22,144</sup>

Baran also employed this methodology to prepare (+)-welwitindolinone A isonitrile **438** from (+)-12-*epi*-fischerindole I isonitrile **434** (Scheme 47),<sup>128</sup> which was treated with tert-butyl hyperchlorite and TFA, resulting in chlorination of the indole ring to afford a chloroimine intermediate **435**. This chloroimine species **434** was intercepted by a water molecule to afford an unstable aminol **435** that eliminated an chloride anion to afford iminium species **436** that undergoes a 1,5-sigmatropic rearrangement reaction to afford both the amide and cyclobutyl ring functionalities of (+)-welwitindolinone A isonitrile **438** in 25% yield.<sup>144</sup> Alternatively, treatment of (+)-12-*epi*-fischerindole I isonitrile **433** with the electrophilic fluorinating agent  $\text{XeF}_2$  resulted in formation of welwitindolinone A isonitrile **438** (*via* fluorinated indole **437** through a similar rearrangement/ring contraction pathway in an improved 44% yield.<sup>22,145</sup>

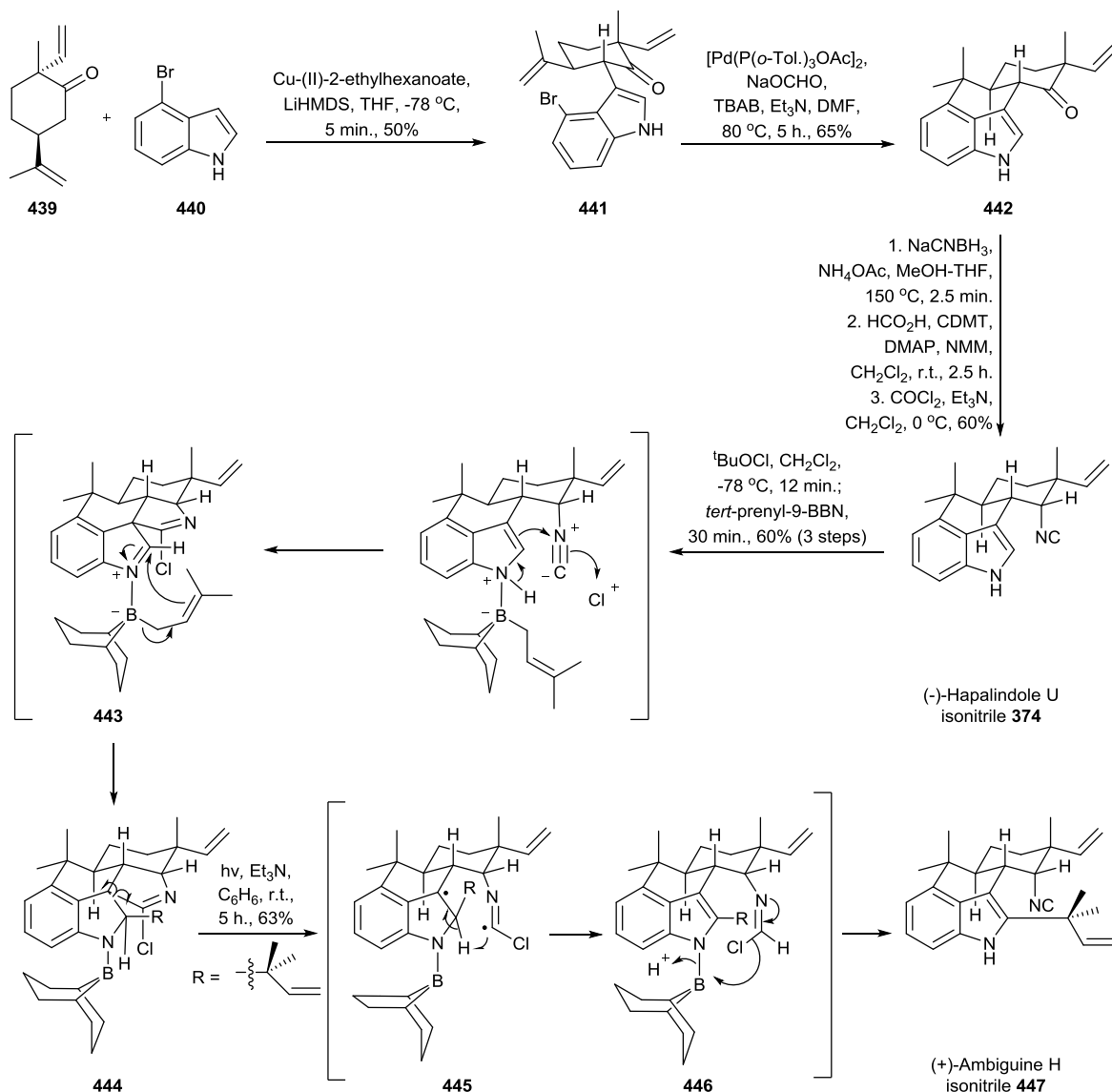




Scheme 47 Baran's syntheses of (+)-welwitindolinone A isonitrile **438**.<sup>22,145</sup>

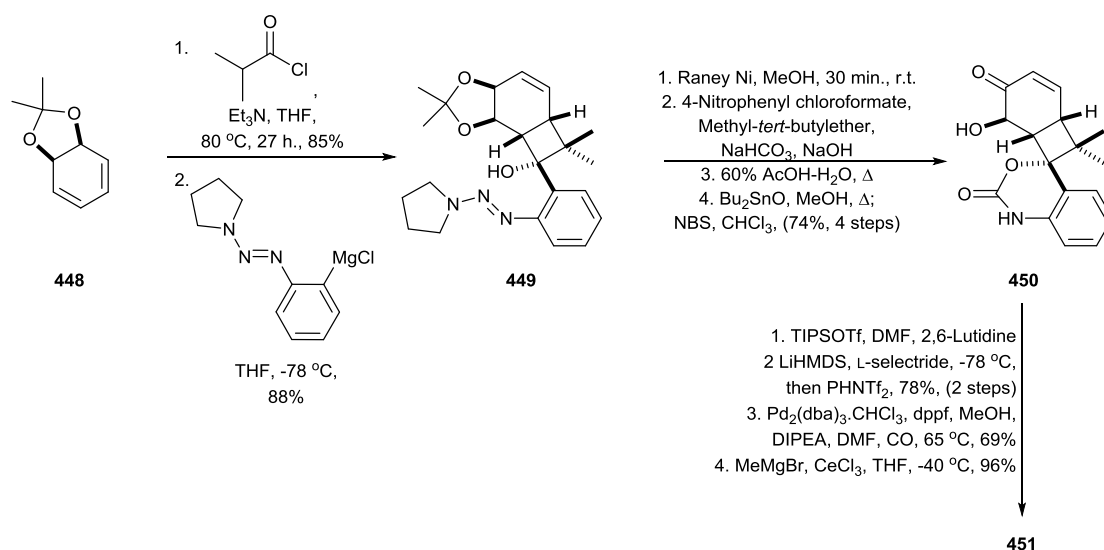
Baran has also reported another 'protecting group free' strategy for the synthesis of the marine isocyanides (-)-hapalindole U isonitrile **374** and (+)-ambiguine H isonitrile **447** (Scheme 48).<sup>145</sup> The lithium enolate of the (*S*)-(+)-carvone derived intermediate **439** was C<sub>3</sub>-arylated with indole **440** in the presence of a Cu(II)-2-ethylhexanoate catalyst to generate indole-ketone **441** as a single diastereomer in 50% yield. Slow addition of [Pd(P(*ortho*-tol)<sub>3</sub>OAc)<sub>2</sub>] to a solution of bromo-indole **441**, NaOCHO, tetra-*n*-butylammonium bromide (TBAB) and Et<sub>3</sub>N in DMF at 80 °C over five hours was successful in facilitating a palladium catalysed cross-coupling reaction to afford the tetracyclic core of ketone **442**. (-)-Hapalindole U isonitrile **374** was then prepared by reductive amination of ketone **442**, followed by *N*-formylation of the resultant amine to generate a formamide (CDMT, HCO<sub>2</sub>H) that was dehydrated using COCl<sub>2</sub> and Et<sub>3</sub>N. Installation of the *tert*-prenyl unit of (+)-ambiguine H isonitrile **447** was achieved *via* reaction of (-)-hapalindole U isonitrile **374** with *tert*-butyl hyperchlorite and prenyl 9-BBN-H, which generated an imino-borane species **443** that underwent a [3,3]-rearrangement reaction that resulted in transfer of the *tert*-prenyl group to its indole ring to afford chloroimide **444**. Exposure of chloroimide **444** to light in the presence of Et<sub>3</sub>N then resulted in a Norrish-type radical fragmentation reaction to afford diradical species **445** that underwent intramolecular

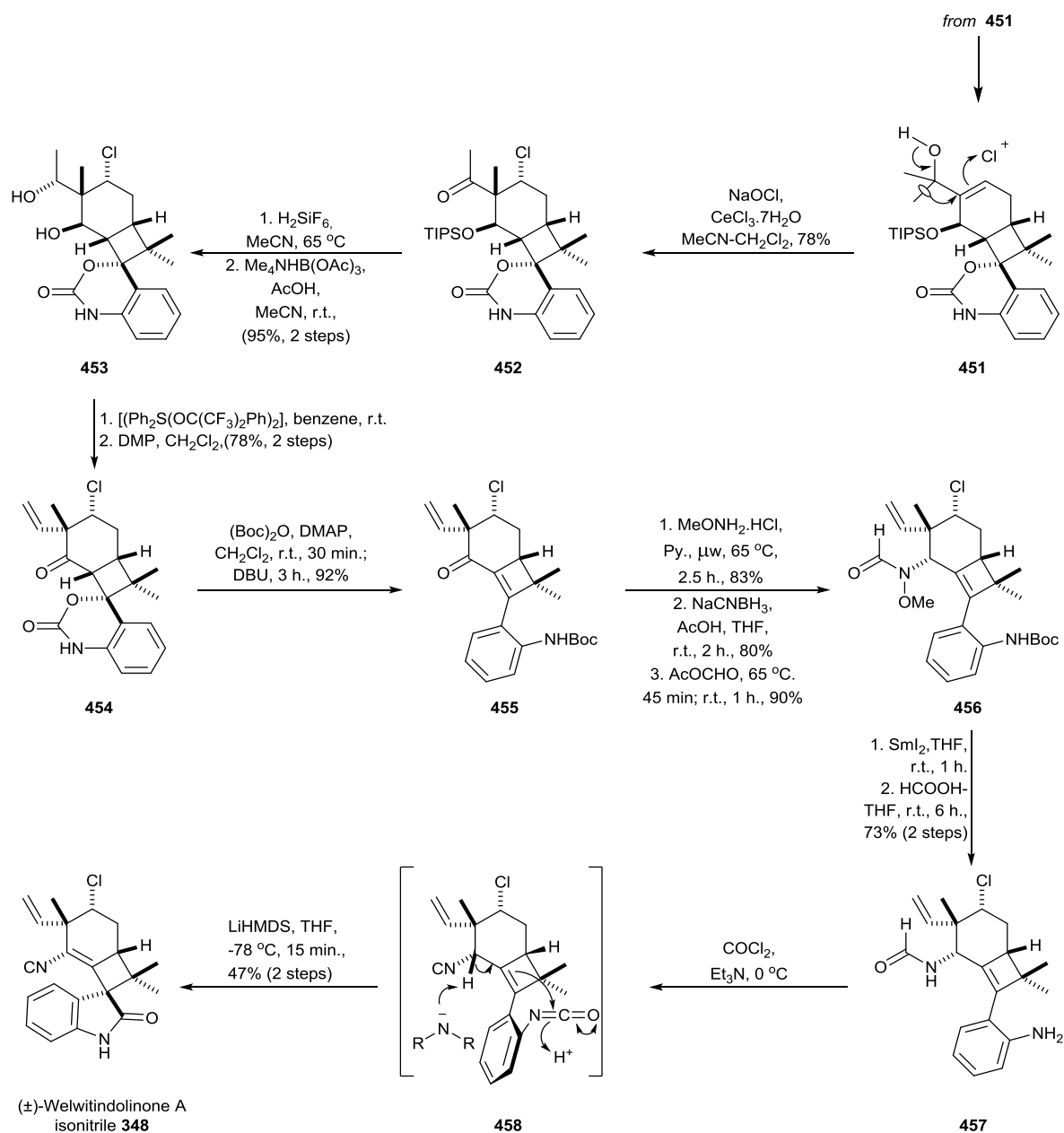
radical hydrogen atom abstraction to afford a chloroimine **446** that eliminated chloride *in situ* to generate the isocyanide fragment of (+)-ambiguine H isonitrile **447**.<sup>145</sup>

Scheme 48 Baran's synthesis of (+)-ambiguine H isonitrile **447** via (-)-hapalindole U isonitrile **374**.<sup>145</sup>

Wood and co-workers have also reported the synthesis of ( $\pm$ )-welwitindolinone A isonitrile **348** (Scheme 49).<sup>146–148</sup> Diene **448** underwent a highly stereo and regioselective [2+2] cycloaddition reaction with dimethyl ketene to afford a tricyclic ketone, which was reacted with Grignard reagent to afford the cyclobutanol fragment of phenyl triazene **449**. Phenyl triazene **449** was then converted into oxazolidin-2-one **450** *via* a four step protocol involving: (i) reduction of the triazene with Raney nickel/H<sub>2</sub> to afford an amino group; (ii) protection of the amino-alcohol group as an oxazolidin-2-one; (iii) acid catalysed acetonide hydrolysis; (iv) selective oxidation of the allylic alcohol functionality using Bu<sub>3</sub>SnO and NBS. The alcohol group of **450** was then

protected as its triisopropylsilyl (TIPS) group, and the resultant enone treated with LiHMDS (to protect the oxazolidin-2-one as its lithium amide), followed by conjugate reduction with L-Selectride and trapping of the resultant enolate with *N*-phenyltriflimide to afford an enol triflate intermediate. This enol triflate was then subjected to a Pd(0)-catalysed carbonylation reaction in the presence of MeOH to afford an ester that was reacted with excess MeMgBr/CeCl<sub>3</sub> to afford tertiary alcohol **451**. Treatment of alcohol **451** with NaOCl/ CeCl<sub>3</sub> resulted in formation of a chloronium intermediate that underwent a semi-pinacol rearrangement reaction to afford a single keto diastereomer **452**. *O*-Silyl deprotection of ketone **452** was followed by stereoselective reduction of the ketone group to afford a diol **453** whose least-hindered alcohol group was selectively dehydrated *via* treatment with Martin's sulfurane reagent [(Ph<sub>2</sub>S(OCF<sub>3</sub>)<sub>2</sub>Ph)<sub>2</sub>], with its remaining alcohol group then being oxidised to afford ketone **454**. Ketone **454** was then reacted with (Boc)<sub>2</sub>O in the presence of DMAP resulting in *N*-Boc protection of its oxazolidin-2-one group, followed by DBU mediated elimination of CO<sub>2</sub> from the oxazolidin-2-one ring to afford an *N*-Boc-aniline **455**. Reaction of ketone **455** with *O*-methylhydroxylamine gave an oxime that was reduced with NaCNBH<sub>3</sub> to stereoselectively afford an *N*-OMe-amine that was treated with acetic formic anhydride to afford *N*-OMe-formamide **456**. The *N*-O bond of **456** was reductively cleaved *via* treatment with SmI<sub>2</sub>, with subsequent formic acid mediated *N*-Boc deprotection affording formamide **457**. A mixture of phosgene/Et<sub>3</sub>N was then employed to access both the isocyanide and isocyanate fragments of the cyclobutene intermediate **458**, which cyclised on exposure to LiHMDS, thus generating the vinyl isocyanide and indan-2-one fragments of welwitindolinone A isonitrile **348**.<sup>146–148</sup>

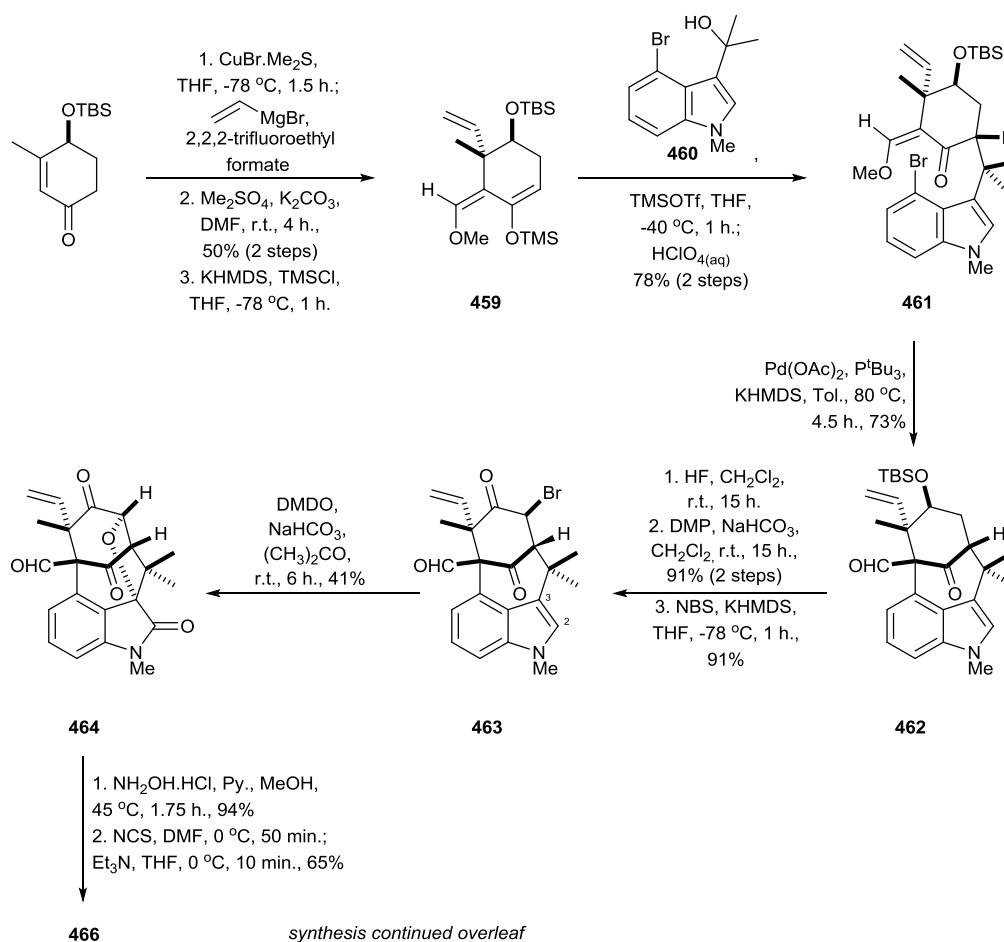




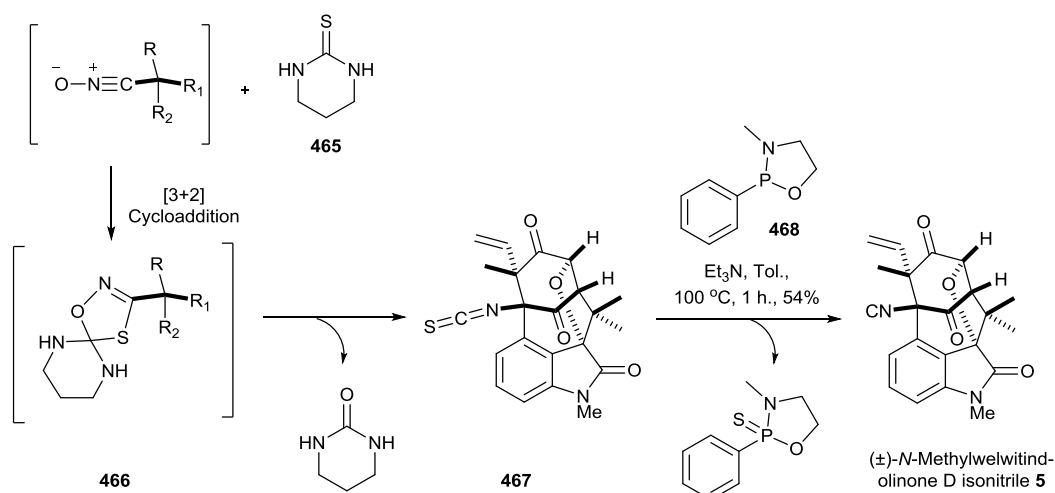
Scheme 49 Wood's synthesis of (±)-welwitindolinone A isonitrile **348**.<sup>146–148</sup>

*N*-Methylwelwitindolinone D isonitrile **5** is known to demonstrate antifungal properties, and the potential to overcome P-glycoprotein-mediated drug resistance in human carcinoma cells. In 2011, Rawal and co-workers developed an approach for the synthesis of (±)-*N*-methylwelwitindolin-2-one **5** that interestingly did not proceed *via* a formamide intermediate.<sup>149</sup> In their synthesis (Scheme 50), trimethylsilyl triflate mediated alkylative coupling of silyl enol ether **459** with a carbocationic species derived from the tertiary alcohol fragment of *N*-methylindole **460** afforded ketone **461** as a single diastereomer. A palladium catalysed intramolecular enolate  $\alpha$ -arylation cross coupling reaction was then used to

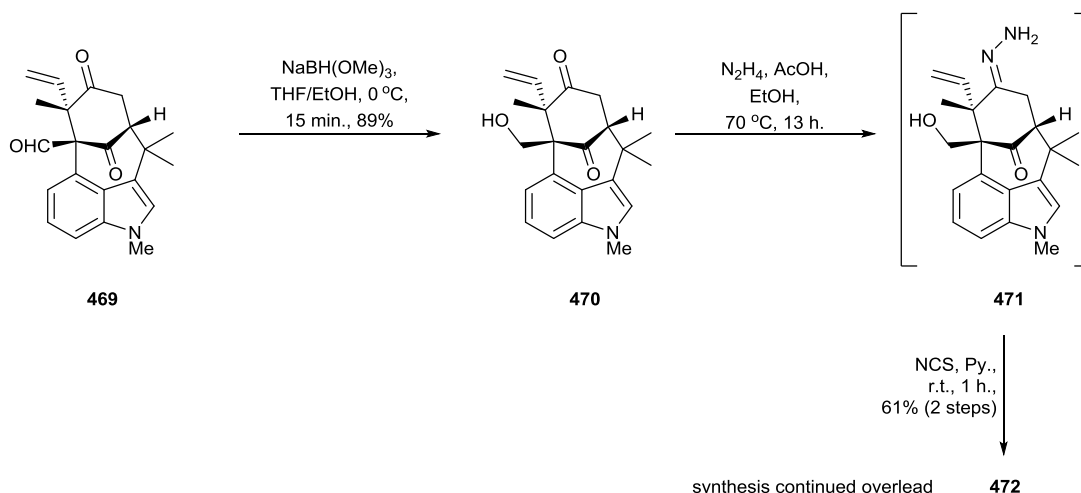
construct the cyclic [4.3.1] scaffold of aldehyde **462**. Aldehyde **462** was transformed into  $\alpha$ -bromoketone **463** over three steps involving: (i) *O*-TBS deprotection; (ii) DMP oxidation; (iii)  $\alpha$ -bromination of the keto group *via* treatment with potassium bis(trimethylsilyl)amide KHMDS and NBS. Treatment of indole **463** with DMDO and NaHCO<sub>3</sub> in DMSO resulted in stereoselective oxidation at the C<sub>2</sub>- and C<sub>3</sub>-positions of the indole ring, with concomitant intramolecular displacement of its bromide by a C<sub>3</sub>-hydroxyl group to afford the cyclic ether bridge of a new tetrahydrofuran ring system. Treatment of the resultant aldehyde **464** with hydroxylamine afforded an oxime intermediate that was transformed into isothiocyanate **467** *via* treatment with NCS, Et<sub>3</sub>N and the cyclic thiourea **465** in 65% yield. This reaction is thought to proceed *via* formation of a nitrile oxide species **466** *in situ* which then undergoes a [3+2] cycloaddition reaction with the cyclic thiourea **465** to afford an unstable 1,4,2-oxathiazoline species **466** that spontaneously decomposes to afford the isothiocyanate functionality of **467**. Treatment of isothiocyanate **467** with oxazaphospholidine **468** resulted in desulphurisation to generate the isocyanide functionality of ( $\pm$ )-*N*-methylwelwitindolin-2-one D isonitrile **5** in 54% yield.<sup>149</sup>



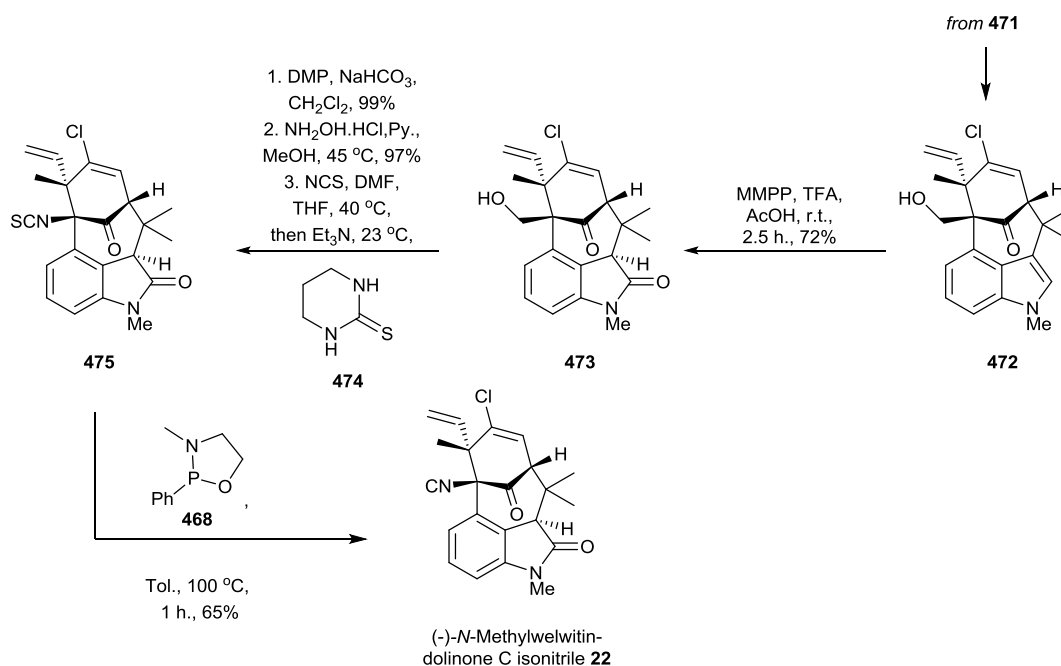



 Scheme 50 Rawal's synthesis of (±)-*N*-methylwelwitindolinone D isonitrile **5**.<sup>149</sup>

Rawal and co-workers also reported the synthesis of (-)-*N*-methylwelwitindolinone C isonitrile **22** using diketo-aldehyde **469** as a key intermediate that had been used previously in their synthesis of *N*-methylwelwitindolinone D isonitrile **5** (Scheme 51).<sup>150</sup> Aldehyde **469** was selectively reduced using sodium trimethoxyborohydride to afford alcohol **470** whose ketone group was reacted with hydrazine to afford hydrazone **471**, which was then treated with NCS to afford the vinyl chloride **472**. Vinyl chloride **472** was then treated with the mild oxidant magnesium monoperoxyphthalate (MMPP) to afford oxindole **473**, which was then oxidised with DMP to afford an aldehyde that was converted into *N*-methylwelwitindolinone C isonitrile **22** over three steps (*via* isothiocyanate **475**) using conditions previously established for their synthesis of (-)-*N*-methylwelwitindolinone D isonitrile **5**.<sup>150</sup>



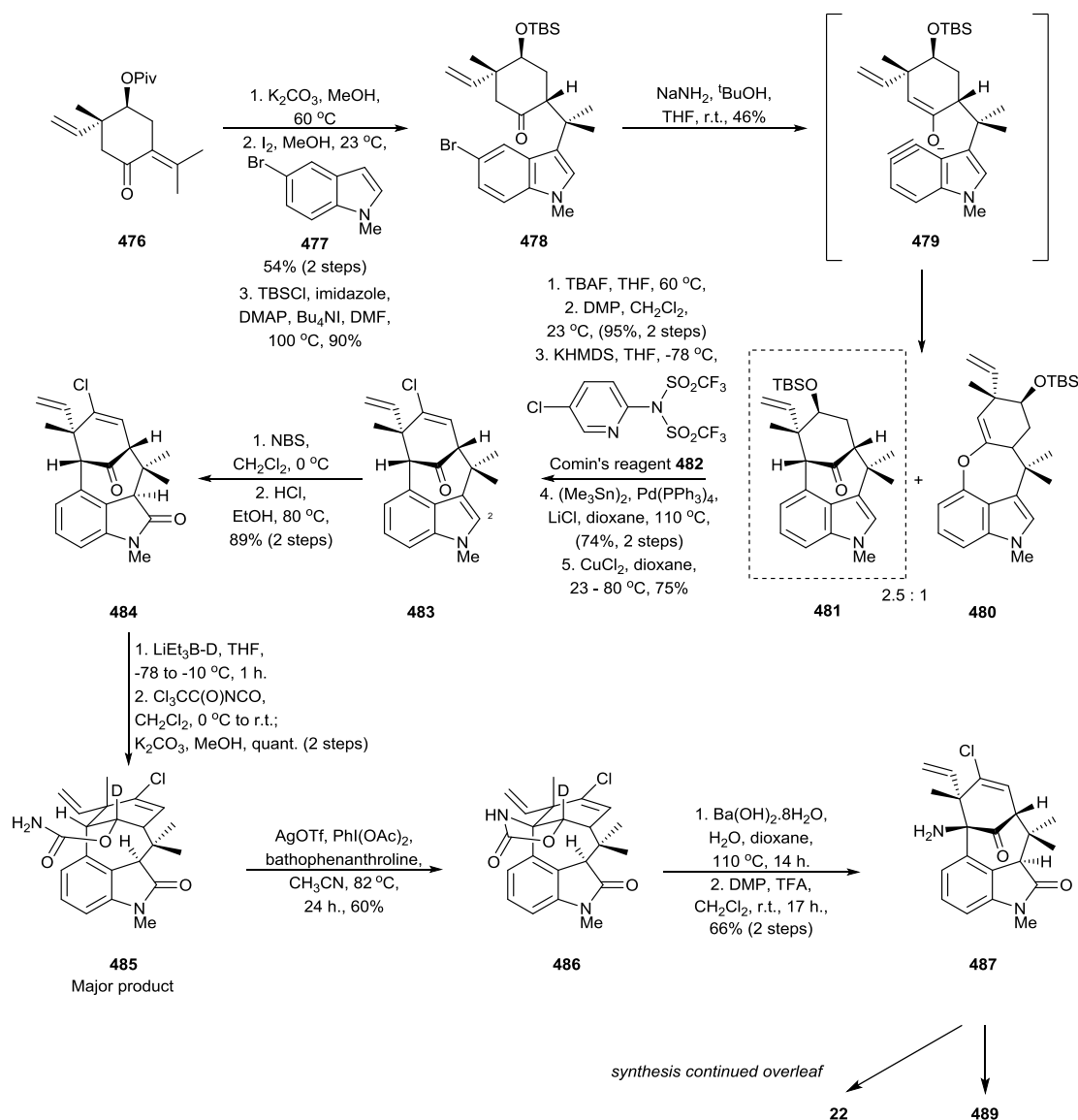
synthesis continued overload

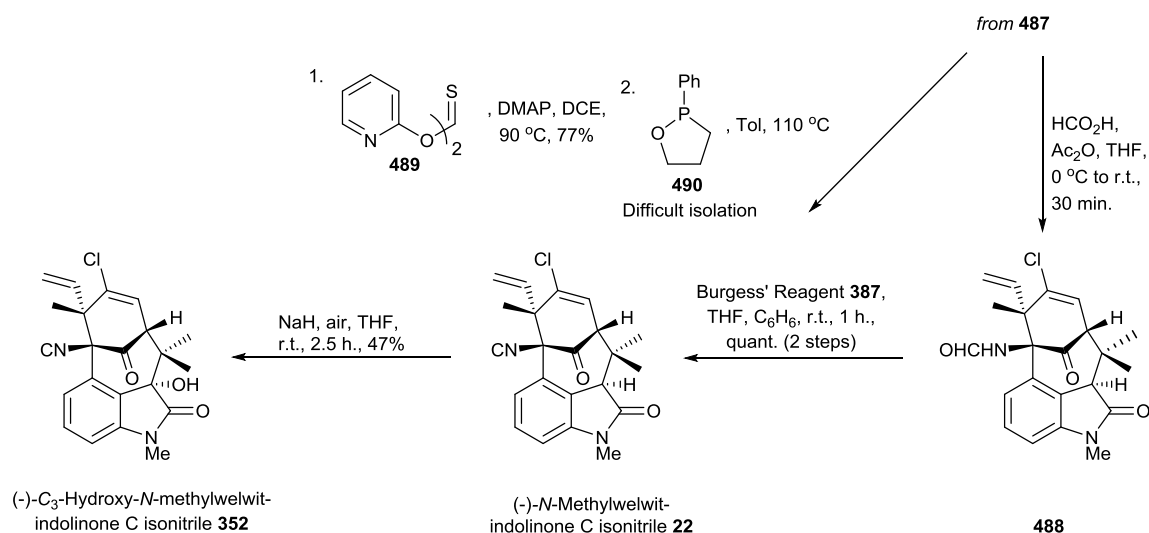


Scheme 51 Rawal's synthesis of (-)-*N*-methylwelwitindolinone C isonitrile **22**.<sup>150</sup>

A stereoselective synthesis of (-)-*N*-methylwelwitindolinone C isonitrile **22** has also been reported by Garg and co-workers (Scheme 52),<sup>151</sup> starting from carvone derived ketone **476** that was transformed into bromoindole **478** over 3 three steps involving (i) methanolysis of the pivaloyl ester; (ii) iodine catalysed intermolecular alkylation of indole; (iii) *O*-TBS protection.<sup>152</sup> Treatment of bromoindole **478** with sodium amide resulted in intramolecular cyclisation of its derived sodium enolate onto an indolyne fragment (*via* intermediate **479**) to give a 2.5 : 1 mixture of ketone **481** and the seven-membered cyclic *O*-aryl ether **480**. Ketone **481** was purified by chromatography and then converted into vinyl chloride **483** over five steps involving: (i) *O*-TBS deprotection; (ii) oxidation of the resultant alcohol with DMP; (iii) vinyl triflate formation from treatment of a ketone enolate with Comin's reagent **482**; (iv) palladium catalysed stannylation of the vinyl triflate; (v) copper catalysed chlorination of vinyl stannane.<sup>153</sup> Treatment of vinyl chloride **483** with NBS resulted in C<sub>2</sub>- bromination to afford a bromoindole that was then hydrolysed to afford indan-2-one **484**. Reduction of the ketone functionality of indan-2-one **484** with LiEt<sub>3</sub>B-D resulted in a deuterated alcohol that was then converted into carbamate **485** *via* sequential treatment with trichloroacetylisocyanate and potassium methoxide. This enabled an elegant deuterium kinetic isotope effect to be used to control the regioselectivity of an intramolecular nitrene C-H insertion reaction that was initiated by treatment of carbamate **485** with AgOTf, PhI(OAc)<sub>2</sub> and bathophenanthroline to afford oxazolidin-2-one **486** in 60% yield. The oxazolidin-2-one fragment of **487** was then hydrolysed with Ba(OH)<sub>2</sub> to afford an amine **487** whose secondary alcohol group was oxidised

into its corresponding ketone with DMP. Amine **487** was then *N*-formylated using acetic formic anhydride to afford formamide **488** that was subsequently dehydrated using Burgess' reagent **387** to generate (-)-*N*-methylwelwitindolinone C isonitrile **22**. Amine **487** could also be converted into *N*-methylwelwitindolinone C isonitrile **22** in lower yield *via* treatment with *O,O*-di(pyridinyl-2-yl)carbonothioate **489** and DMAP in dichloroethane to afford an isothiocyanate that was then desulphurised *via* treatment with oxazaphospholidine **490**. Subsequent exposure of a solution of (-)-*N*-methylwelwitindolinone C isonitrile **22** and NaH in THF to air resulted in selective bridgehead oxidation to afford (-)-*C*<sub>3</sub>-hydroxy-*N*-methylwelwitindolinone C isonitrile **352**.<sup>152</sup>

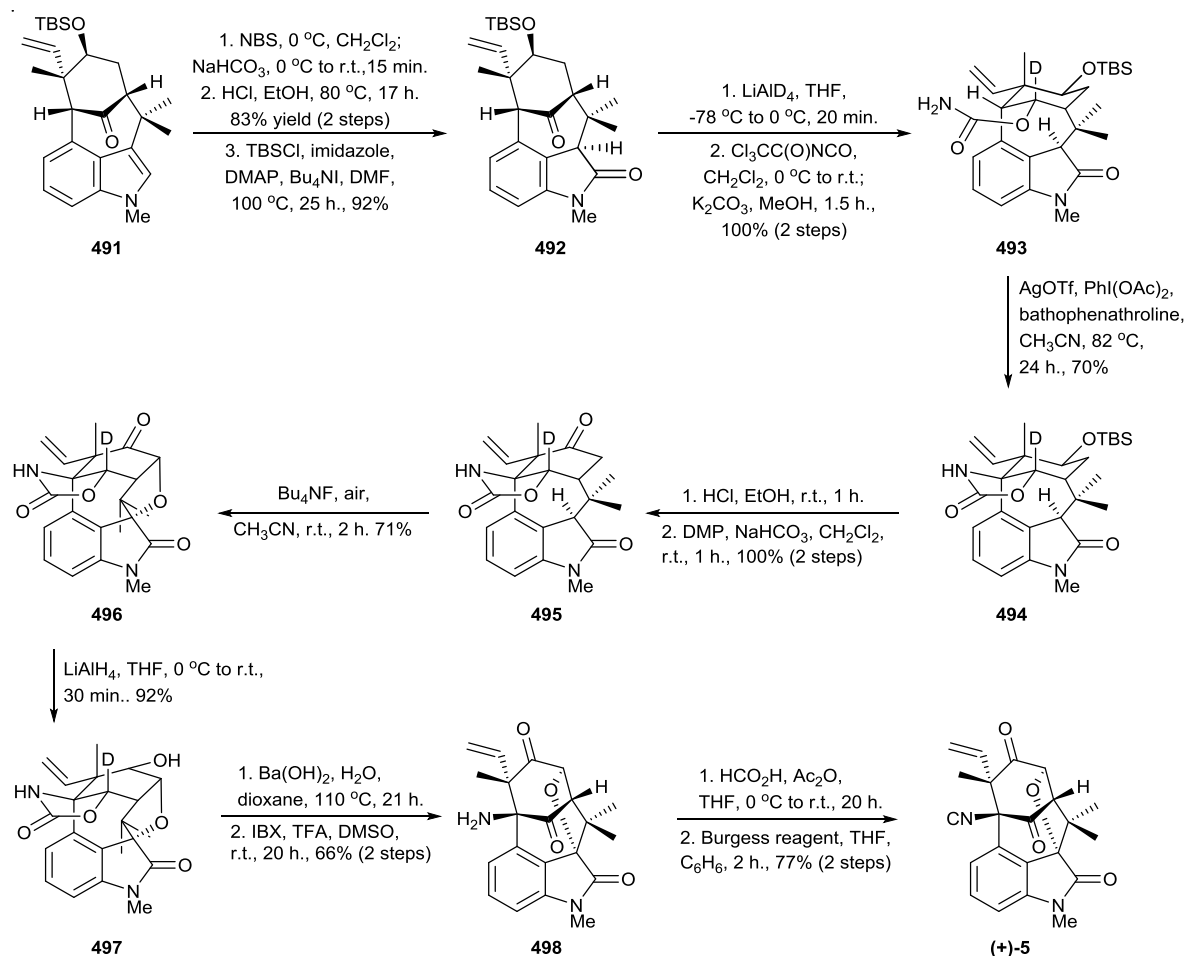




Scheme 52 Garg's syntheses of (-)-N-methylwelwitindolinone C isonitrile **22** and (-)-C<sub>3</sub>-hydroxy-N-methylwelwitindolinone C isonitrile **350**.<sup>152</sup>

Garg was also able to achieve the enantiospecific synthesis of (+)-N-methylwelwitindolinone D isonitrile **5** by first converting previously synthesised ketone **481** into oxoindole **492** via a one pot-pot procedure that involved oxidation with NBS and hydrolysis using HCl (Scheme 53).<sup>154</sup> The substrate had to be resilylated *in situ* to afford oxoindole **492** which subsequently underwent deuteride reduction to give an alcohol that was then carbamoylated to form primary amide **493**. Secondary amide **494** was afforded upon silver promoted nitrene insertion reaction of **493** with  $\text{PhI}(\text{OAc})_2$  and bathophenanthroline, with subsequent hydrolysis/oxidation reactions yielding ketone **495**. The ether functionality of **496** was realised upon exposure of ketone **495** to TABF in the open air, with the resulting furan species **496** being reduced to alcohol **497** using  $\text{LiAlH}_4$ . Barmium hydroxide facilitated hydrolysis afforded a diol, which was subsequently oxidised with IBX to afford *bis*-ketone **498**. The isocyanide functionality of **5** was generated upon formylation of amine **498** with formic acid and acetic anhydride, providing a formamide that was subsequently dehydrating using Burgess reagent **387** to afford (+)-N-methylwelwitindolinone D isonitrile **5**.<sup>154</sup>

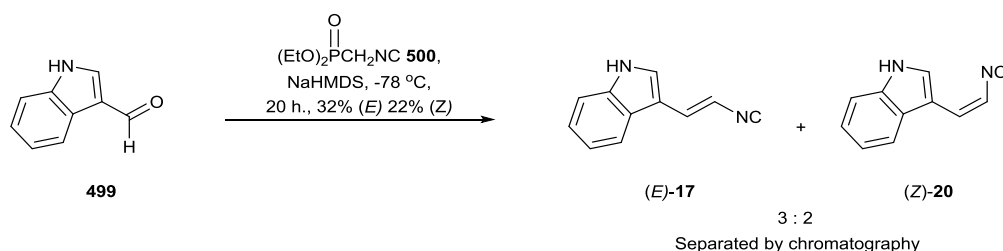
### Methodology for the synthesis of isocyanide containing natural products



Scheme 53 Garg's synthesis of (+)-*N*-methylwelwitindolinone D isonitrile **5**.<sup>154</sup>

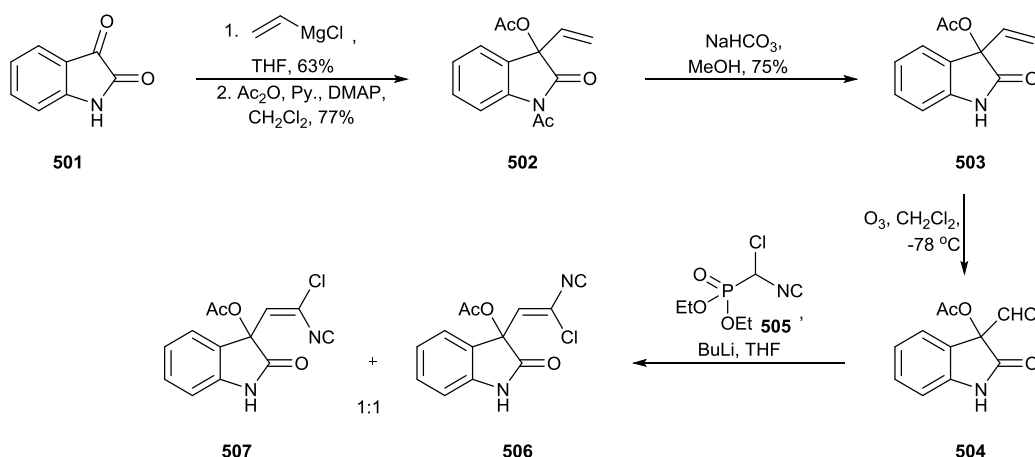
### 1.4 Syntheses of miscellaneous isocyanide natural products

(*E*)-3-Indole vinyl-isocyanide **17** and (*Z*)-3-indole vinyl-isocyanide **20** are antibiotic metabolites that exhibit strong antimicrobial properties in low concentration against *Escherichia coli*, *Bacillus subtilis* and *Mucor muhei* TU 284.<sup>19,155</sup> Hoppe and Schöllkopf reported the synthesis of these 3-indole vinyl-isocyanides *via* Horner-Wadsworth-Emmons reaction of the anion of diethyl isocyanomethylphosphonate **500** with 3-indole carboxaldehyde **499** using sodium bis(trimethylsilyl)amide (NaHMDS) as a base (Scheme 54). Under these conditions a 3 : 2 mixture of the vinylogous isocyanides (*E*)-**17** and (*Z*)-**20** isomers was produced that were separated by column chromatography.<sup>155</sup>



Scheme 54 Schöllkopf's synthesis of antibiotics (E)-17 and (Z)-20.<sup>155</sup>

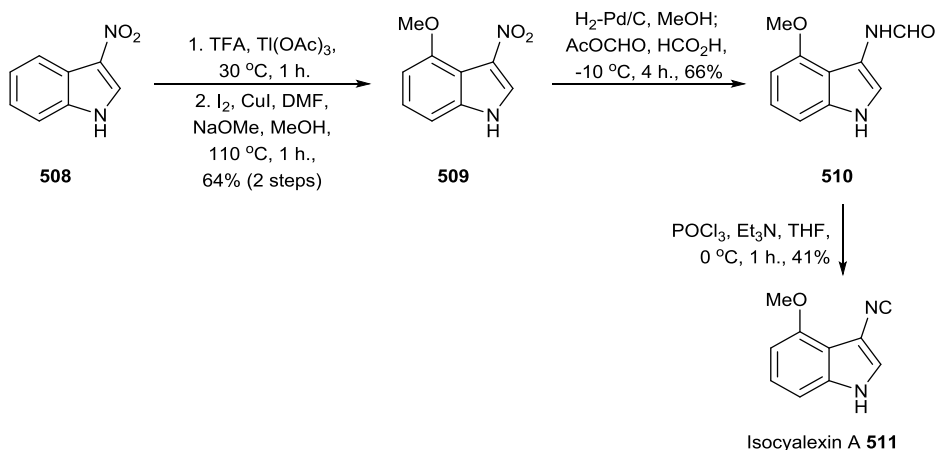
An isocyanide antibiotic indisocin **507** containing a 1-chlorovinylisocyanide moiety that was isolated from the actinomycete strain MG323-hF2 was synthesised *via* addition of vinyl magnesium bromide to isatin **501** to afford an alcohol that was then *bis*-acetylated to afford the acetate and acetamide fragments of indole **502** (Scheme 55).<sup>156</sup> Selective methanolysis of the *N*-acetyl bond afforded an acetate **503** that was ozonised to afford aldehyde **504** that underwent Horner-Wadsworth-Emmons reaction with the anion of diethyl chloromethylisocyanophosphonate **505** to afford a 1 : 1 mixture of (Z)- and (E)-  $\alpha,\beta$ -unsaturated- $\alpha$ -chloro-isocyanides **506** and **507** that could be separated *via* chromatography.<sup>156</sup>



Scheme 55 Tatsuta's synthesis of indisocin **507**.<sup>156</sup>

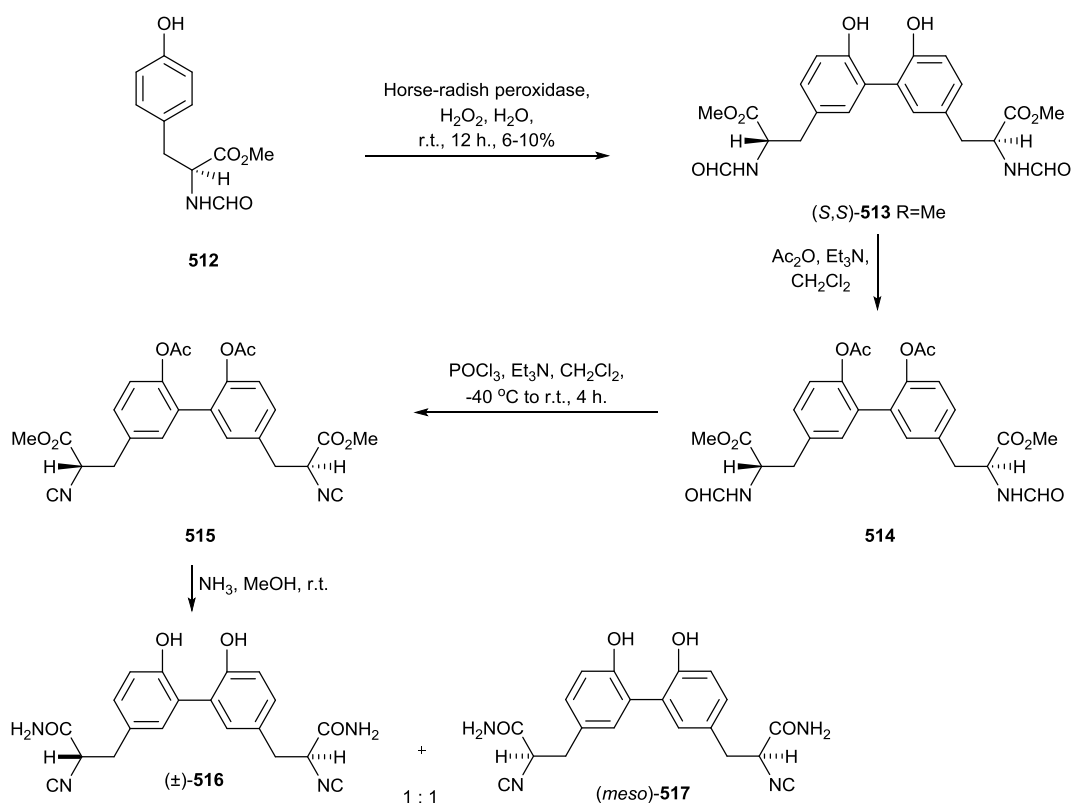
Isocyaalexin A **511** is the first known isocyanide extracted from a plant, which was isolated after UV-irradiation of rutabaga root slices, followed by incubation in the dark, organic extraction and fractionation.<sup>157</sup> Biological testing against four fungal root pathogens revealed antifungal activity against *A. brassicicola*, *L. maculans*, *R. solani*, *S. sclerotiorum*, suggesting that isocyaalexin A **511** is produced as a phytoalexin to protect the plant against root pathogens.<sup>157</sup> In 2012, Pedras and Yaya described the total synthesis of isocyaalexin A **511** (Scheme 56), employing 3-nitroindole **508** as a starting material for a sequence of thallation-iodination-methoxylation reactions with good regiocontrol to install the C<sub>4</sub>-methoxy group of indole **509**.

This indole intermediate **509** was then hydrogenated to give an amine intermediate that was *N*-formylated with acetic formic anhydride to give formamide **510**, with subsequent Et<sub>3</sub>N mediated dehydration in the presence of POCl<sub>3</sub>/Et<sub>3</sub>N affording isocyaalexin A **511** in 41% yield.<sup>157</sup>



Scheme 56 Pedras' synthesis of isocyaalexin A **511**.<sup>157</sup>

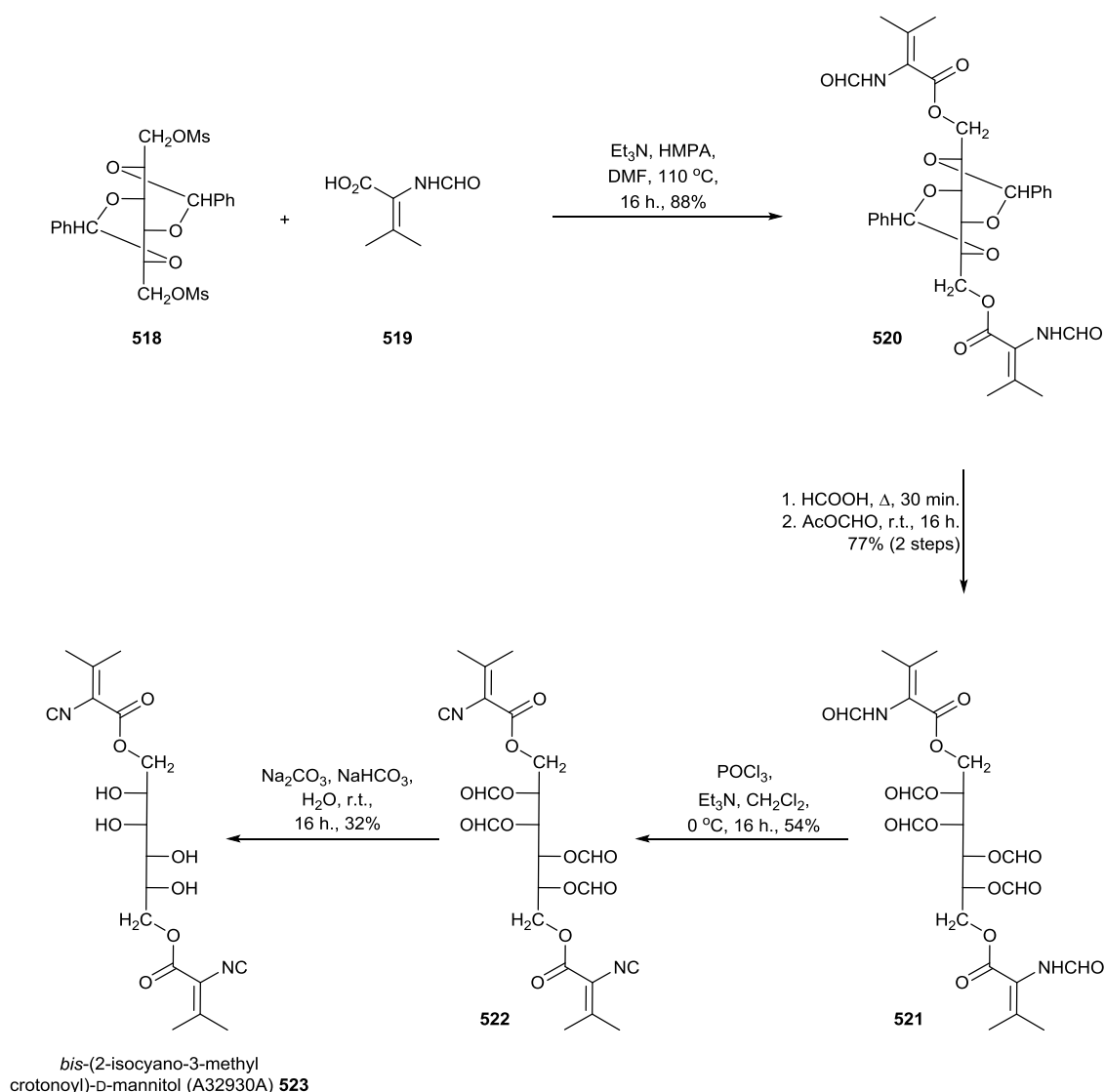
Factors 5 **516** and 6 **517** have been identified as two primary components of hazimycin metabolites produced by *Pseudomonas* sp. SCC 1411, a bacterium isolated from soil that demonstrated *in vitro* and *in vivo* activity against Gram-positive and Gram-negative bacteria, and *in vitro* activity against yeasts and dermatophytes. Wright and co-workers used POCl<sub>3</sub> as a formamide dehydrating agent in their 1982 synthesis of hazimycin factor 5 (±)-**516** and hazimycin factor 6 (*meso*)-**517**, which were shown to rapidly interconvert in the presence of base. For their synthesis (Scheme 57),<sup>158</sup> *N*-formyl-L-tyrosine methyl ester **512** was oxidatively homocoupled using horse-radish peroxidase in low 6-10% yield to form the required *bis*-aryl dimer **513**, whose phenolic groups were then protected as their *bis*-*O*-acetates **514**. Standard POCl<sub>3</sub>/Et<sub>3</sub>N mediated formamide dehydration conditions were then used to efficiently generate the isocyanide fragments of **515**. Tandem ammonolysis of the phenolic acetate groups and the two ester functionalities of **515** was achieved *via* treatment with methanolic ammonia, with concomitant racemisation of both stereocentres occurring to generate a mixture of (±)-**516** and (*meso*)-**517** in a 1 : 1 ratio.<sup>158</sup> Achab and Velay subsequently reported a formal synthesis of hazimycin based on palladium (0) catalysed coupling of an aryl iodide with an aryl stannane to afford *bis*-formamide **514** in 56% yield (scheme 69).<sup>159</sup>



Scheme 57 Wright's synthesis of hazimycin factor 5 (±)-**516** and hazimycin factor 6 (*meso*)-**517**.<sup>158, 159</sup>

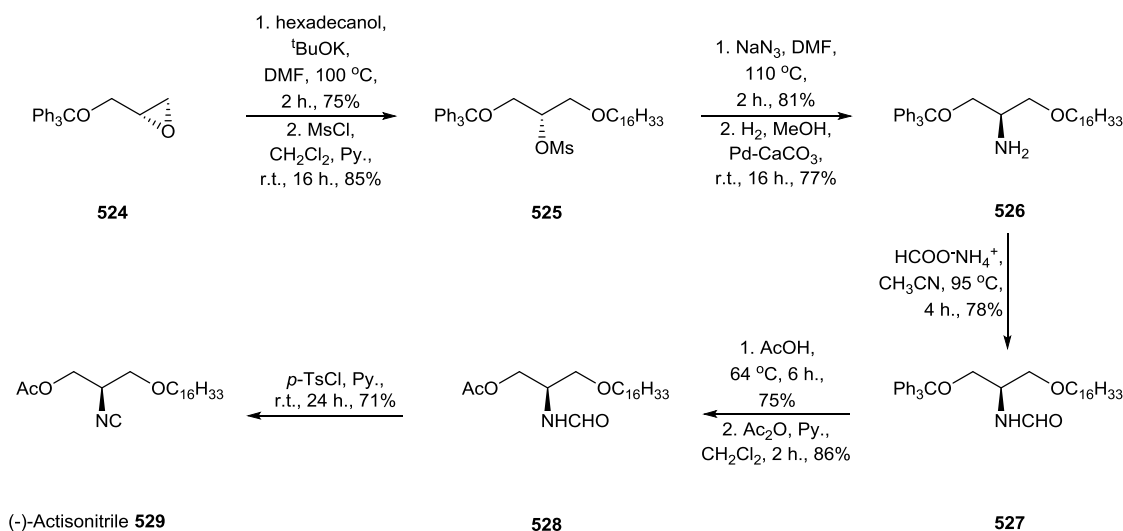
The sugar derived isocyanide antibiotic A32930A **523** was prepared *via* nucleophilic displacement of the *bis*-*O*-benzylidene acetal protected mannitol *bis*-mesylate **518** with two equivalents of the carboxylate anion of vinyl formamide **519** to afford *bis*-formamide ester **520** (Scheme 58). The benzylidene groups of *bis*-formamide **520** were then globally deprotected *via* heating in formic acid, and the resultant tetrol performylated using acetic formic anhydride. The *bis*-formamide functionality of **521** was then dehydrated using  $\text{POCl}_3/\text{Et}_3\text{N}$  to produce *bis*-isocyanide **522**, whose remaining *O*-formyl groups were then hydrolysed under basic conditions ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_{3(\text{aq})}$ ) to afford the natural product *bis*-2-isocyano-3-methylcrotonoyl)-D-mannitol (A32930A) **523**.<sup>160</sup>





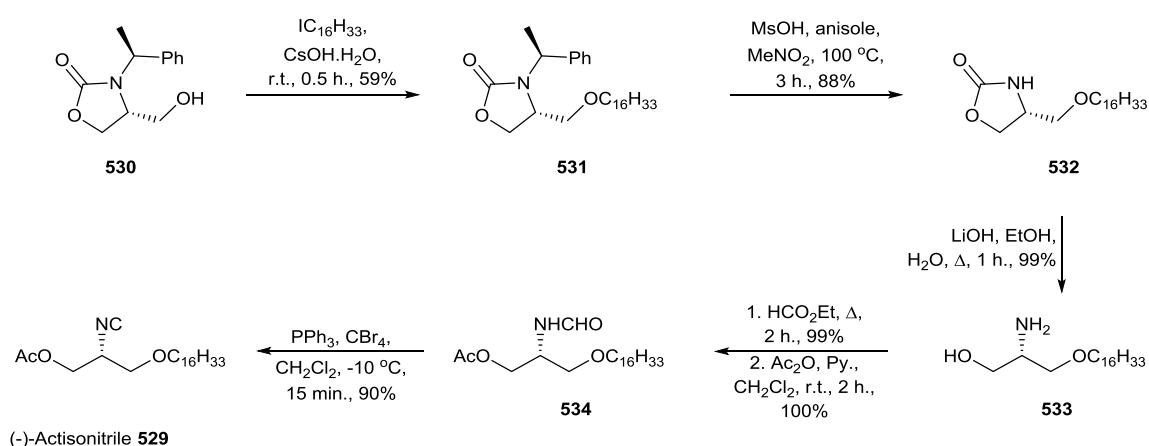
Scheme 58 Schollkopf's synthesis of *bis*-(2-isocyano-3-methylcrotonoyl)-D-mannitol (A32930A) **523**.<sup>160</sup>

Gavagnin and co-workers have reported the synthesis of (-)-actisonitrile **529**, a 1,3-propanediol ether based lipid derived isocyanide isolated from the nudibranch *Actinocyclus papillatus*, that demonstrated cytotoxicity against non-tumour H9c2 rat cardiac myoblast cells ( $\text{IC}_{50} = 23 \mu\text{M}$ ).<sup>161</sup> For their synthesis of (-)-actisonitrile **529** (Scheme 59),<sup>161</sup> chiral trityl-epoxide **524** was ring-opened using the alkoxide of hexadecanol to afford a secondary alcohol intermediate that was mesylated to generate ether **525**. Reaction of ether (S)-**525** with sodium azide resulted in nucleophilic substitution with clean inversion of configuration, followed by hydrogenation of the resultant azide to afford amine (R)-**526**, which was transformed into formamide **527** by treatment with ammonium formate. Acid catalysed removal of the trityl group of formamide **527** was followed by acetylation of the resultant alcohol group to afford a formamide **528** that was dehydrated *via* treatment with tosyl chloride in pyridine to afford (-)-actisonitrile **529**.<sup>161</sup>



Scheme 59 Gavagnin's synthesis of (-)-actisonitrile **529**.<sup>161</sup>

A second synthesis of (-)-actisonitrile **529** was reported by Sugiyama *et al.* with oxazolidinone **530** being *O*-alkylated *via* treatment with 1-iodohexadecane and caesium hydroxide to afford ether **531** (Scheme 60).<sup>162</sup> Ether **531** was then debenzylated *via* treatment with MsOH and anisole in nitromethane to afford *NH*-oxazolidinone **532** that upon hydrolysis with LiOH afforded 1,2-amino-alcohol **533**. 1,2-Amino-alcohol **533** was *N*-formylated using ethyl formate and the alcohol functionality of the resultant formamide acetylated with  $\text{Ac}_2\text{O}$ /pyridine to afford acetate **534**, which upon exposure to  $\text{PPh}_3/\text{CBr}_4$  was dehydrated to yield (-)-actisonitrile **529**.<sup>162</sup>



Scheme 60 Sugiyama's synthesis of (-)-actisonitrile **529**.<sup>162</sup>

## 1.5 Conclusion

Since the discovery of xanthocillin as the first naturally occurring isocyanide, a diverse range of isocyanide containing natural products have been isolated from marine and terrestrial sources that exhibit a broad range of potent biological activities. The biological activities and structural complexity of many of these isocyanides has resulted in some impressive syntheses, however a significant number of biologically active isocyanide containing natural products **535-542** remain to be synthesised (Figure 18).<sup>163-172</sup> Compilation of this review has revealed that a finite number of strategies currently exist to install the reactive isocyanide group into structurally complex molecules (Figure 19 Common strategies for formamide dehydrationFigure 19), with a general preference for installing the reactive isocyanide group at a late stage of a synthetic protocol. By far, the most popular strategy employed for introducing the isocyanide functionality into a natural product target is by dehydration of its corresponding formamide, which is invariably generated *via* *N*-formylation of its corresponding primary amine. However, a few noteworthy alternatives for the introduction of isocyanide group have been explored, based on the *N*-alkylation of nucleophilic cyanide species with cationic intermediates, desulfurisation of isothiocyanates and Horner-Wadsworth-Emmons reactions of  $\alpha$ -isocyano-containing phosphonates. From a synthetic perspective, the syntheses of many structurally challenging terpene derived isocyanide natural products are particularly noteworthy, with many ingenious strategies having been developed for the construction of their complex polycyclic backbones in a stereoselective manner. In this respect, there is little doubt that the isocyanide class of natural products will continue to provide inspiration for the development of future methodology at the cutting edge of synthesis.

## Methodology for the synthesis of isocyanide containing natural products

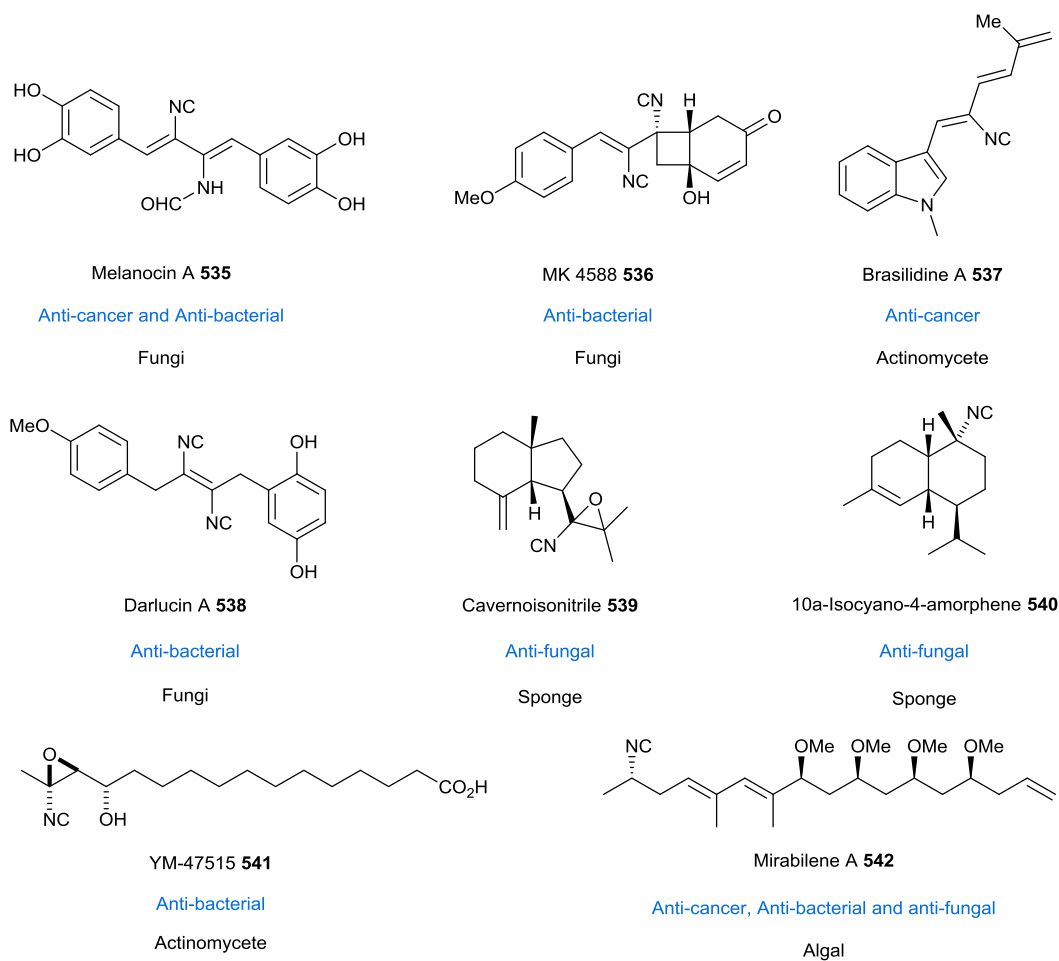


Figure 18 Biologically important isocyanides **535-542** that have yet to be synthesised.<sup>163–168,170–172</sup>

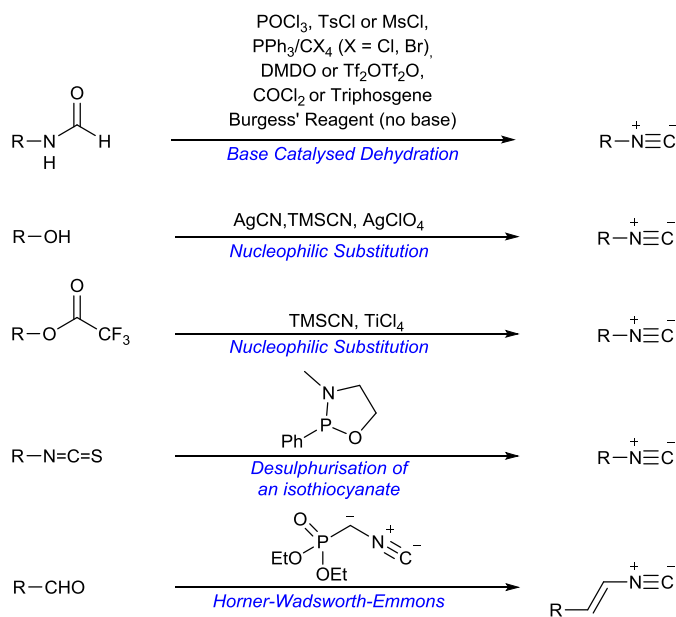


Figure 19 Common strategies for formamide dehydration.

## 2 The development of an *E*-selective Horner-Wadsworth-Emmons protocol for the synthesis of phenol-vinyl isocyanide

### 2.1 Insects as a source of Natural Products

A variety of organisms found in nature are capable of producing chemical structures (natural products) that exhibit potent biological activity. To date, a variety of blockbuster drugs have been developed from lead compounds produced by microorganisms found in plants and various members of the animal kingdom. However, natural products produced by microorganisms found within insects are currently an under-explored resource.<sup>173</sup> Insects, which are found in nearly all environments, represent at least half of the known living organisms on this planet, making them the most diverse subset of the animal population. Insects emerged over 400 million years ago and this has facilitated the evolution of parasites (e.g. bacteria) that exclusively colonise insects as hosts.<sup>174</sup> These highly evolved microorganisms express secondary metabolic pathways that produce toxins (insecticides) with targeted, pathogenic behaviour towards their host. The chemical structures (Figure 20) of these metabolites can be regarded as a potentially huge library of structurally diverse compounds for screening for pharmaceutical applications.<sup>173,174</sup>

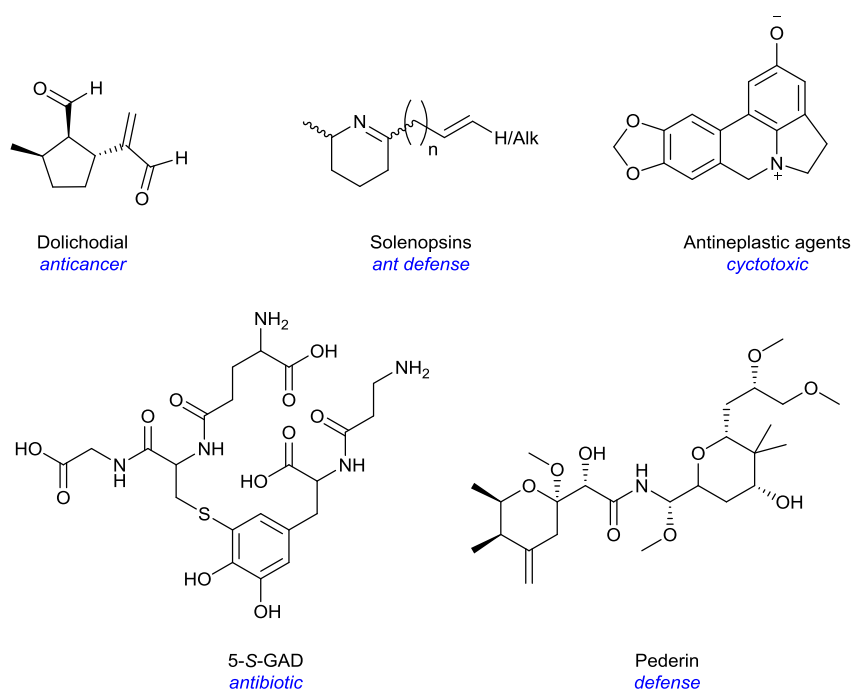


Figure 20 Structures of insect natural products and their associated biological properties.<sup>175</sup>

Consequently, the remainder of this thesis will concern itself with the synthesis and biological evaluation of the vinyl-isocyanide containing secondary metabolites; phenol vinyl-isocyanide **542**, Rhabduscin **543** and Byelyankacin **544**, which are produced by the insect pathogens *Photorhabdus* and *Xenorhabdus* bacteria that exist in symbiosis with heterorhabdus nematodes (Figure 21).

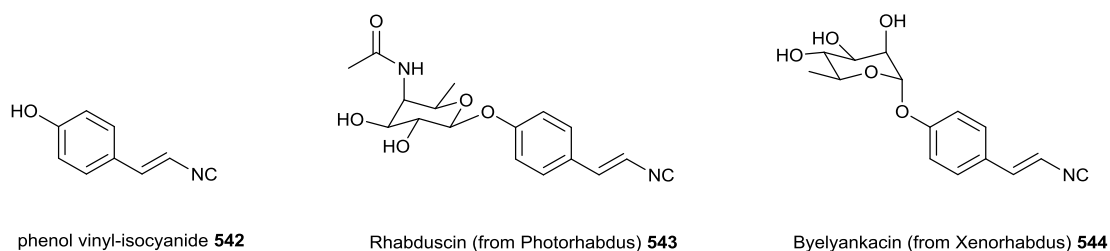


Figure 21 Isocyanide derived natural products from *Photorhabdus* and *Xenorhabdus* bacteria.

## 2.2 Life cycle of heterorhabdus nematodes.

*Photorhabdus* and *Xenorhabdus* bacteria live within the *Heterorhabditis* nematode in a mutualistic relationship, where they exist in trilateral symbiosis to parasitize insect larvae.<sup>174,176–178</sup> In the soil, juvenile nematodes host *Photorhabdus* and *Xenorhabdus* in their gut, carrying these organisms with them as they penetrate their larval insect prey directly, or enter through other natural orifices. Regurgitation by the nematodes then occurs, releasing between 50 to 200 bacterial cells into the host's open blood system, with *Photorhabdus* and *Xenorhabdus* now switching to a pathogenic state, where they start to produce a series of secondary metabolites. These include phenol vinyl-isocyanide **542**, Rhabduscin **543** and Byelyankacin **544** which combine to facilitate the host's destruction by suppressing its immune system, facilitating bacterial reproduction by enabling the nematodes to feed and grow. Curiously, the bacteria is also known to continue producing these isocyanide derived natural products long after the host's death, which may enable it to effectively defend and compete against other microbial competitors for the larval carcass. To complete the life cycle, the nematode then returns to its infective juvenile stage, and exits the insect carcass in search of new prey, whilst still carrying the *Photorhabdus* in its gut (Figure 22).<sup>174,176–178</sup>

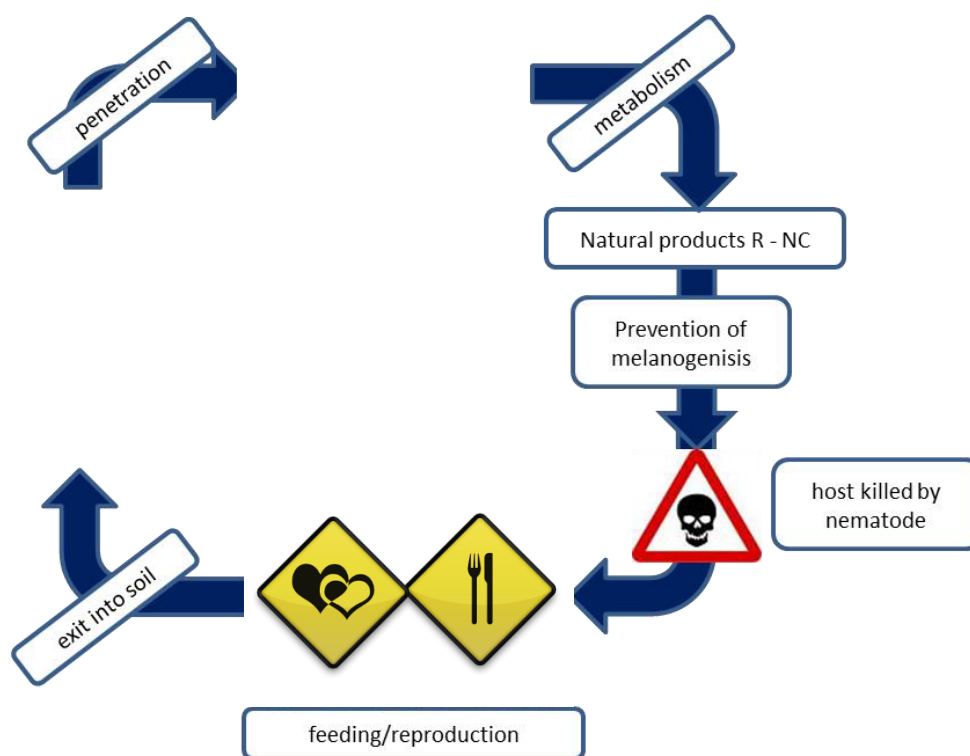


Figure 22 Lifecycle of heterorhabdus nematode.

### 2.3 Inhibition of tyrosinases by Rhabduscin and Byelyankacin

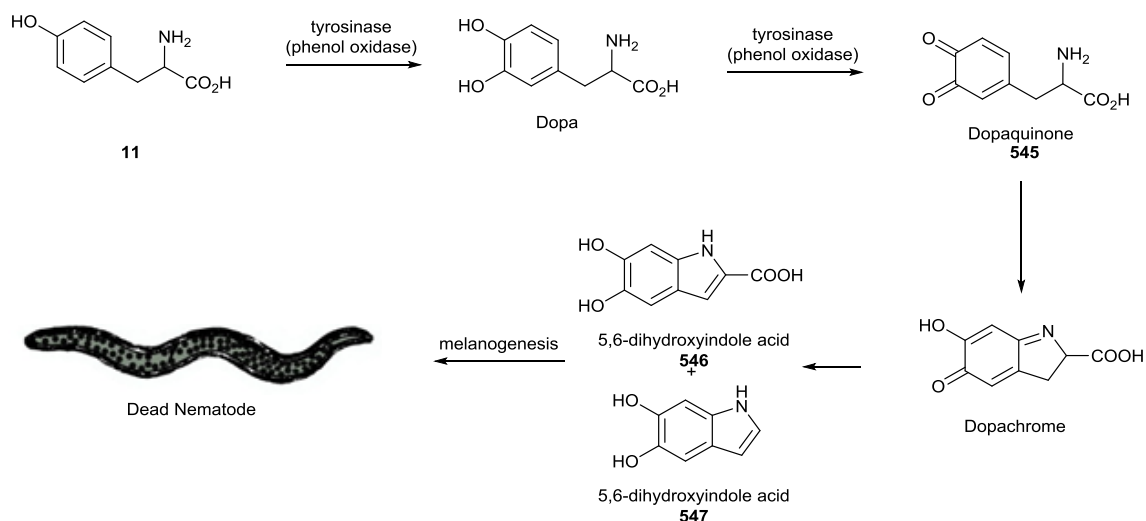
The secondary metabolites Rhabduscin **543**, Byelyankacin **544** and phenol vinyl-isocyanide **542** have been shown to exhibit low nanomolar inhibitory activity against mushroom tyrosinases and wax moth larvae phenol oxidase (Table 1).<sup>179</sup>

Table 1 Enzyme inhibition ( $IC_{50}$ )(M) of secondary metabolites produced by the bacteria

Metabolite	Mushroom tyrosinase inhibition	Phenol oxidase inhibition
Rhabduscin <b>543</b>	$15.1 \times 10^{-9}$	$64.1 \times 10^{-9}$
Byelyankacin <b>544</b>	$37.1 \times 10^{-9}$	$184.9 \times 10^{-9}$
Phenol-vinylisocyanide <b>542</b>	$7.9 \times 10^{-9}$	$61.7 \times 10^{-9}$

It is known that insects employ tyrosinase enzymes as part of their innate immune response to invading pathogens to catalyse the oxidation of phenol intermediates for the formation of melanin polymer (melanogenesis). The role of the phenol oxidase is to convert tyrosine **11** into dopaquinone **545** that then acts as a reactive intermediate to generate 5,6-

dihydroxyindoles **546** and **547** that then polymerise to trap invading species in an impermeable coat of melanin, thus encasing the invading organism and suppressing its pathogenic actions (Scheme 61).<sup>180</sup>



Scheme 61 Involvement of tyrosinase as a key enzyme in melanogenesis.

This knowledge enabled Clardy and co-workers to propose that *Photorhabdus* and *Xenorhabdus* deploy the vinyl-isocyanide metabolites **542**, **543** and **544** as inhibitors of host tyrosinase oxidases that are responsible for melanin formation. This prevents the nematodes from being encased in melanin polymer, thus enabling them to continue to effectively parasitise their larval host.

Phenol oxidases are copper containing monooxygenases that are known to bind isocyanide ligands to form Cu(I)-complexes, and as a consequence it has been proposed that the isocyanide carbon of the metabolites were binding to copper in the active site of these tyrosinases as part of the inhibitory response.<sup>181</sup> A similar metal binding event has been proposed to explain the bioactivity of the natural isocyanide kalihinol F **548** (a triisocyanide derived terpene) towards zebra fish. In this case, it has been shown that binding of the isocyanide fragment to copper transport proteins present in developing zebra fish, results in noticeable defects in pigment formation, haematopoiesis, and neural development (Figure 23).<sup>182</sup>



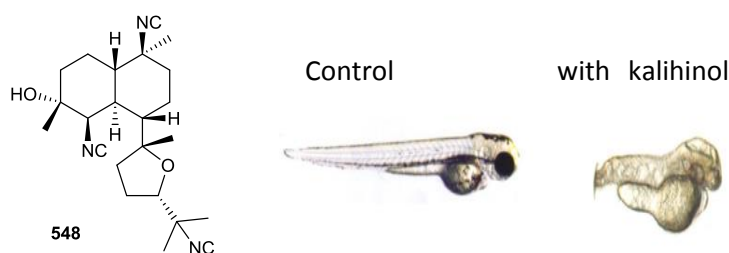


Figure 23 Kalihinol F and its disruption of zebrafish growth *via* chelation to copper transporters.<sup>182</sup>

Clardy and co-workers have provided further information on the biological mode of action of these vinyl-isocyanide natural products by using a *Xenorhabdus* knock out strain (*isnAB* disabled) to prepare a vinyl-azide analogue **549** probe *in vivo* (Figure 24).<sup>179</sup> The azide handle of this analogue **550** was subjected to a copper catalysed click reaction with a biotin-alkyne substrate *in vivo* to afford a triazole adduct **551** containing a biotin fragment. Exposure of this adduct to a streptavidin labelled fluorophore resulted in strong complexation with the biotin fragment of complex **551** *in vivo*. This enabled fluorescence microscopy to be used to reveal that complex **552** was localised at the external cell walls of the bacteria, presented in a location where it could interact directly with the host's bloodstream.

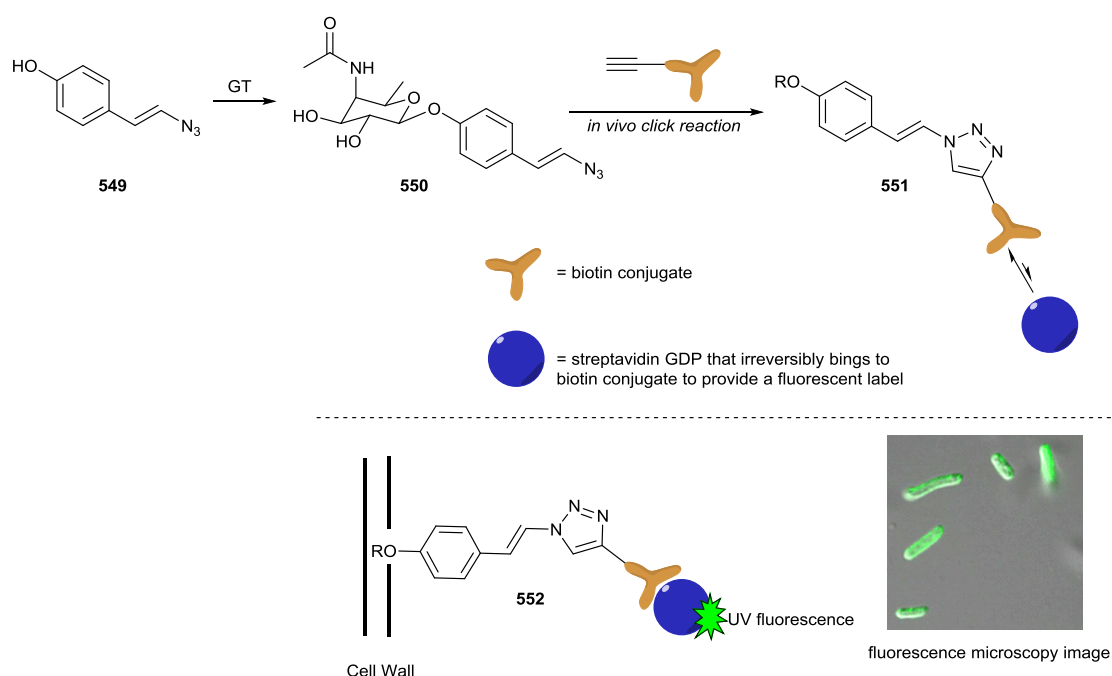


Figure 24 Summary of Clardy's study into cell wall localisation using fluorescent aglycone mimic **549**.

Given this evidence, a working hypothesis to explain the biological activity of these vinyl-isocyanide natural products may be proposed whereby the sugar fragment binds to the cell membrane, localising the isocyanide warhead outside of the bacterial cell-wall (Figure 25). This enables the isocyanide fragment to bind directly to the copper site of the host's tyrosinase enzymes, thus preventing the host's innate immune system from facilitating melanin formation. Examination of the tyrosinase inhibition results presented in Table 1 reveals that the 'free' phenol vinyl-isocyanide **542** exhibits the lowest  $IC_{50}$  values, suggesting that the sugar fragments of Rhabduscin **543** and Byelyankacin **544** do not contribute significantly to their tyrosinase inhibitory activity. This suggests that tyrosinase inhibition *in vivo* may be associated with a hydrolytic glycosidic cleavage event that releases 'free' phenol vinyl-isocyanide **542** from membrane bound Rhabduscin **543** and Byelyankacin **544**.

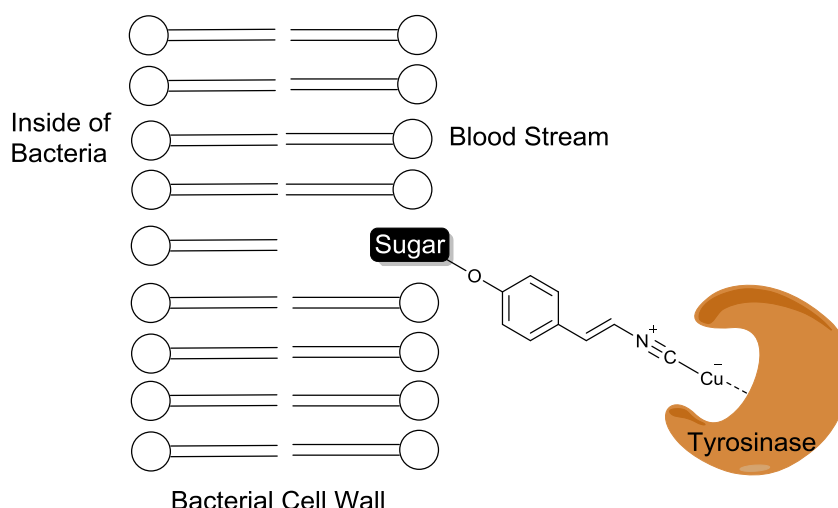


Figure 25 Speculated copper binding and prevention of melanin biosynthesis.

## 2.4 Antibacterial activity of structurally related vinyl isocyanides

Whilst there is now ample evidence that vinyl-isocyanide natural products can inhibit tyrosinases and suppress the host insect innate immune system, their continued production after the insect host has died led us to propose that they may exhibit a potential secondary antibiotic role. This antibiotic activity would enable the bacteria and nematode to effectively compete against other bacterial and fungal organisms for consumption of the insect carcass.

This theory is not without evidence, since two separate studies have previously demonstrated the antibacterial properties of  $\alpha,\beta$ -unsaturated isocyanides. Schöllkopf, in addition to preparing naturally occurring 3-indole vinyl-isocyanides **17** and isomeric antibiotic B371 **20**, prepared a small library of heterocyclic  $\alpha,\beta$ -unsaturated isocyanides.<sup>183</sup> These indole (**553** and **554**) and thiophene (**555**) based substrates were screened for their antibiotic activity using disc diffusion assays and shown to demonstrate antibiotic activity against *E. coli*, *B. subtilis*, and *M. muhei* (Figure 26).

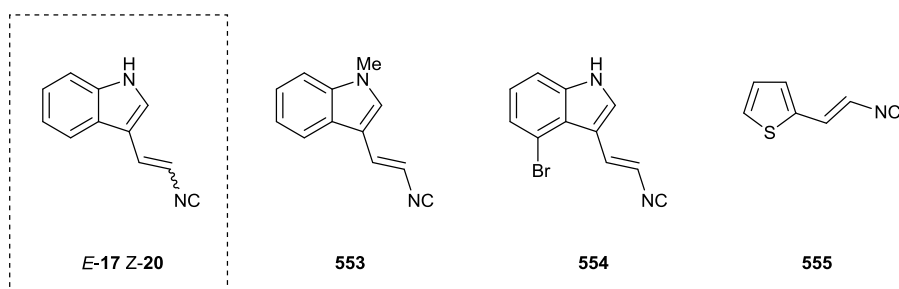


Figure 26 Potent antibiotic heterocyclic isocyanides reported by Schöllkopf.<sup>183</sup>

Following Schöllkopf's pioneering work, a patent was filed in 1987 by a Japanese team investigating the synthesis of the  $\alpha,\beta$ -unsaturated formamide containing natural product, Erbstatin **556**. In addition to screening some formamide analogues, the inventors' also prepared a range of  $\alpha,\beta$ -unsaturated isocyanides (inc **557** and **558**), some of which were reported to exhibit 100 fold more antibiotic activity than their corresponding formamides against *S aureus* and *P. aeruginosa* A3. (Figure 27).

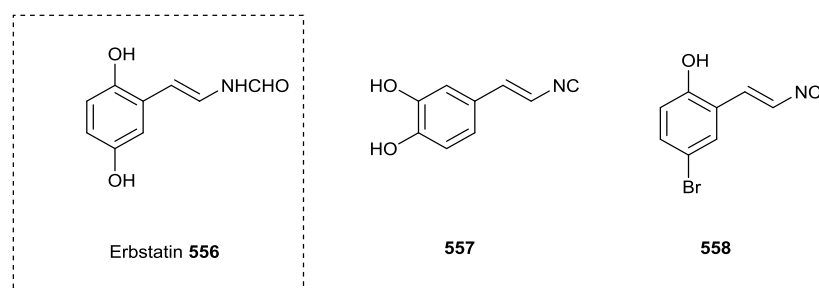


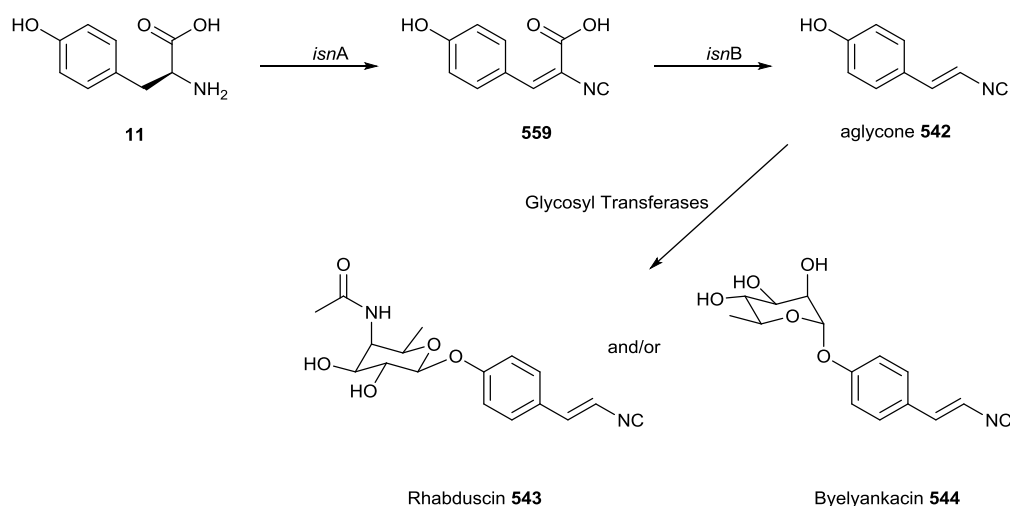
Figure 27 Erbstatin **556** and reported isocyanide analogues with  $\mu$ M antibiotic activities against *S aureus* and *P. aeruginosa* A3.

## 2.5 Project Outline

Since these vinyl-isocyanide natural products are only produced by bacteria in very small amounts, the primary objective of this research program was to devise versatile synthetic methodology to prepare these vinyl-isocyanides for biological evaluation.

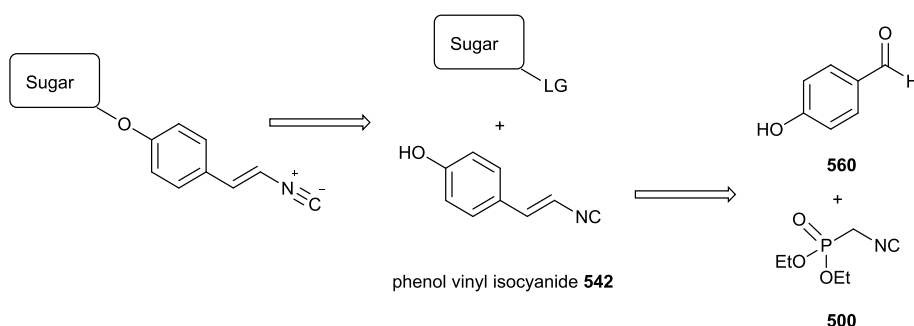
## 2.6 Biosynthesis of Rhabduscin and Byelyankacin

The biosynthetic origins of the isocyanide functionality of the common phenol vinyl isocyanide **542** fragment of these natural products involves the sequential action of two enzymes *isnA* and *isnB* on tyrosine **11** (Scheme 62). The first enzymatic step catalysed by *isnA* involves introduction of the  $\alpha,\beta$ -unsaturated functionality and isocyanide group of intermediate **559**. Labelling studies on the biosynthesis of the related indole isocyanide **17** indicate that the C1-unit of the isocyanide group of intermediate **559** is likely to originate from the C2 atom of ribulose-5-phosphate. *IsnB* then catalyses decarboxylation of intermediate **559** to afford the key aglycone **542**. The sugar residues required for the construction of Rhabduscin **543** and Byelyankacin **544** are then coupled to the aglycone **542** unit through the action of glycosyltransferase (GT) enzymes (Scheme 62).



Scheme 62 Biosynthesis of Rhabduscin **543** and Byelyankacin **544** (via aglycone **542**).

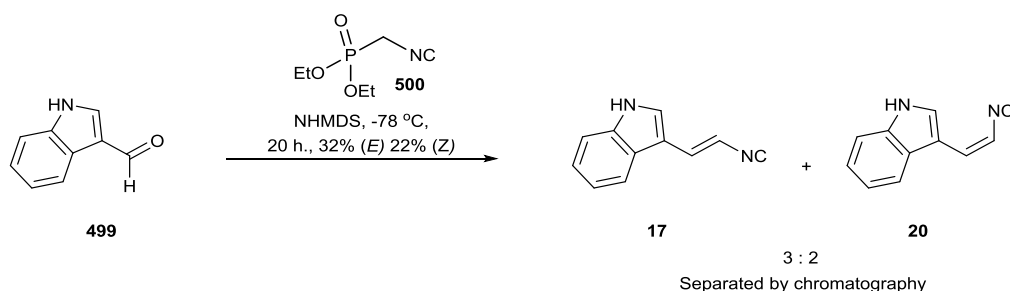
This led us to consider a bio-inspired retrosynthetic analysis of Rhabduscin **543** and Byelyankacin **544** involving coupling the aglycone **542** to an appropriate sugar fragment that was functionalised with an appropriate leaving group at its anomeric position (Scheme 63). Consequently, the challenges encountered in developing a stereoselective synthesis of phenol vinyl-isocyanide (aglycone) **542** via Horner-Wadsworth-Emmons (HWE) reaction of *p*-hydroxybenzaldehyde **560** with the enolate of a suitable phosphonate derivative **500** will be discussed in this chapter. The synthesis of suitable sugar fragments and their coupling with aglycone **542** for the total synthesis of Rhabduscin **543** and Byelyankacin **544** will be discussed in chapter 4.



Scheme 63 Retrosynthesis of Byelyankacin **544**, Rhabduscin **543** and phenol vinyl-isocyanide **542**.

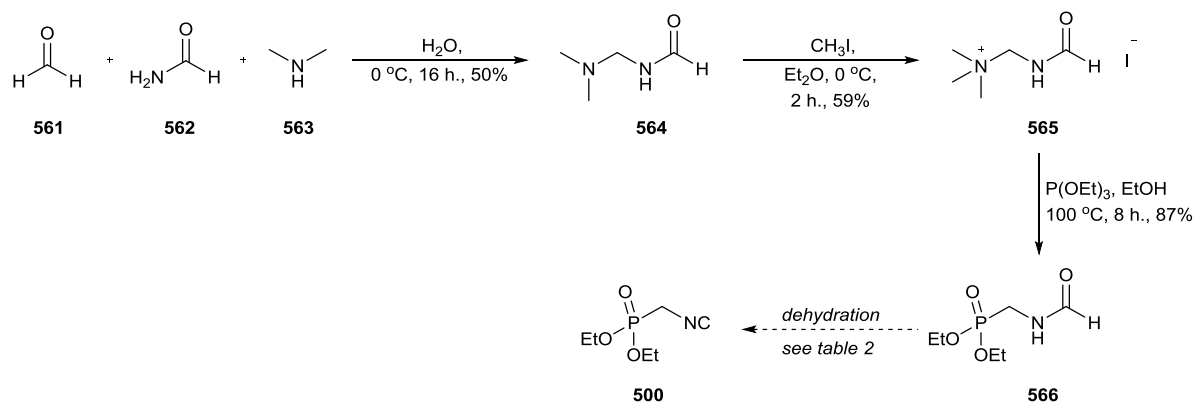
## 2.7 Use of Schöllkopf's reagent for the synthesis of vinyl-isocyanide aglycone **542**.

Schöllkopf and co-workers had already reported the use of isocyanide derived diethyl isocyanomethylphosphonate reagent (PhosMIC **500**) for the synthesis of 3-indole vinyl isocyanide **17** (and antibiotic B371 **20**), via reaction of the phosphonate with carboxaldehyde **499** under typical Horner-Wadsworth-Emmons conditions (Scheme 64).<sup>155</sup> However, their synthesis was not selective for the formation of *E*- $\alpha,\beta$ -unsaturated isocyanide products, with **17** produced as a 3 : 2 mixture with its *Z*- isomer **20**, and subsequently separated by silica gel chromatography.



**Scheme 64** Schöllkopf's synthesis of 3-indole vinyl-isocyanide *E*-17 *Z*-20.

It was therefore envisaged that these conditions could potentially be applied to the synthesis of phenol vinyl-isocyanide **542**, providing the desired aglycone for the synthesis of Byelyankacin **544** and Rhabduscin **543**. Despite the commercial availability of diethyl isocyanomethylphosphonate **500**, it was decided to develop an 'in house' synthesis of this reagent due its high cost and the limited quantities for sale (Scheme 65). In a repeat of a literature synthesis, aqueous dimethylamine **563**, formamide **562** and aqueous formaldehyde **561** underwent a Mannich type condensation reaction to afford amino-formamide **564** that was then stirred with iodomethane at 0 °C to produce its corresponding quaternary amine salt **565**. Having purified this salt by fractional crystallisation, an Arbuzov reaction was then carried out involving refluxing the amino-formamide **565** with triethylphosphite in ethanol to afford the desired phosphonate-formamide **566**, a species that was fully characterised for the first time. Its structure was confirmed by the presence of formamide C-H peaks at 7.80 ppm and a methyl doublet of doublets at 3.50 ppm in the  $^1\text{H}$  NMR spectrum, carbonyl peak at 162 ppm in the  $^{13}\text{C}$  NMR spectrum, a carbonyl stretch in the infra-red spectra and a molecular ion at 218.0575.

Scheme 65 Synthesis of phosphonate-formamide **566**.

Owing to the number of different reagents available for the dehydration of formamides into their corresponding isocyanide, several of the more frequently used dehydrating agents were screened for the transformation of **566** into its corresponding Horner-Wadsworth-Emmons reagent (PhosMIC) **500** (Table 2). For each reaction, the appropriate dehydrating agent was added dropwise to formamide **566** and an excess of triethylamine in dichloromethane at  $-78\text{ }^{\circ}\text{C}$ . These reactions were then allowed to warm up to room temperature overnight, with the reaction then being worked up and the crude product purified by flash column chromatography to afford the desired PhosMIC reagent. As can be seen from the results, the use of methanesulfonyl chloride gave the best yields, consistently affording PhosMIC **500** in around 50% isolated yield. This was not only an improvement on Schöllkopf's  $\text{POCl}_3$  dependent synthesis, the use of a milder dehydration agent also provided a less hazardous work-up. Evidence for PhosMIC's isocyanide functionality was eluded by the presence of a characteristic N-C stretch at  $2121\text{ cm}^{-1}$  and a peak in the carbon NMR spectrum at  $160.8\text{ ppm}$ .

Table 2 Screening of common dehydration reagents for synthesis of **500**.

Entry	Reagent	Yield (%)
1	$\text{POCl}_3$	32
2	TsCl	32
3	$\text{PPh}_3/\text{CBr}_4$	46
<b>4</b>	<b><i>MsCl</i></b>	<b>51</b>

This optimised methodology was then used to prepare PhosMIC **500** on a 5 gram scale, and its use was then trialled for the known synthesis of 3-indole vinyl-isocyanide **17** using conditions previously reported by Schöllkopf and co-workers (Scheme 61). Therefore, the phosphonate carbanion of **500** was generated by treating with 1.2 equivalents of NHMDs in THF at -78 °C, followed by addition of indole 3-carboxaldehyde **499** under anhydrous conditions, to give a 3 : 2 mixture of *E/Z* 3-indole vinyl-isocyanide **17/20** isomers. The poor selectivity of this HWE reaction was confirmed by analysis of the  $^1\text{H}$  NMR spectrum of the crude reaction product which revealed well resolved resonances for their  $\alpha$ -alkene protons at  $\delta$  5.7 ppm (*Z*,  $J$  = 8.6Hz) and  $\delta$  6.3 ppm (*E*,  $J$  = 14.2Hz) (Figure 28). However, the mixture of *E* and *Z* isomers could subsequently be separated by chromatography to afford each geometric isomer in its pure form.

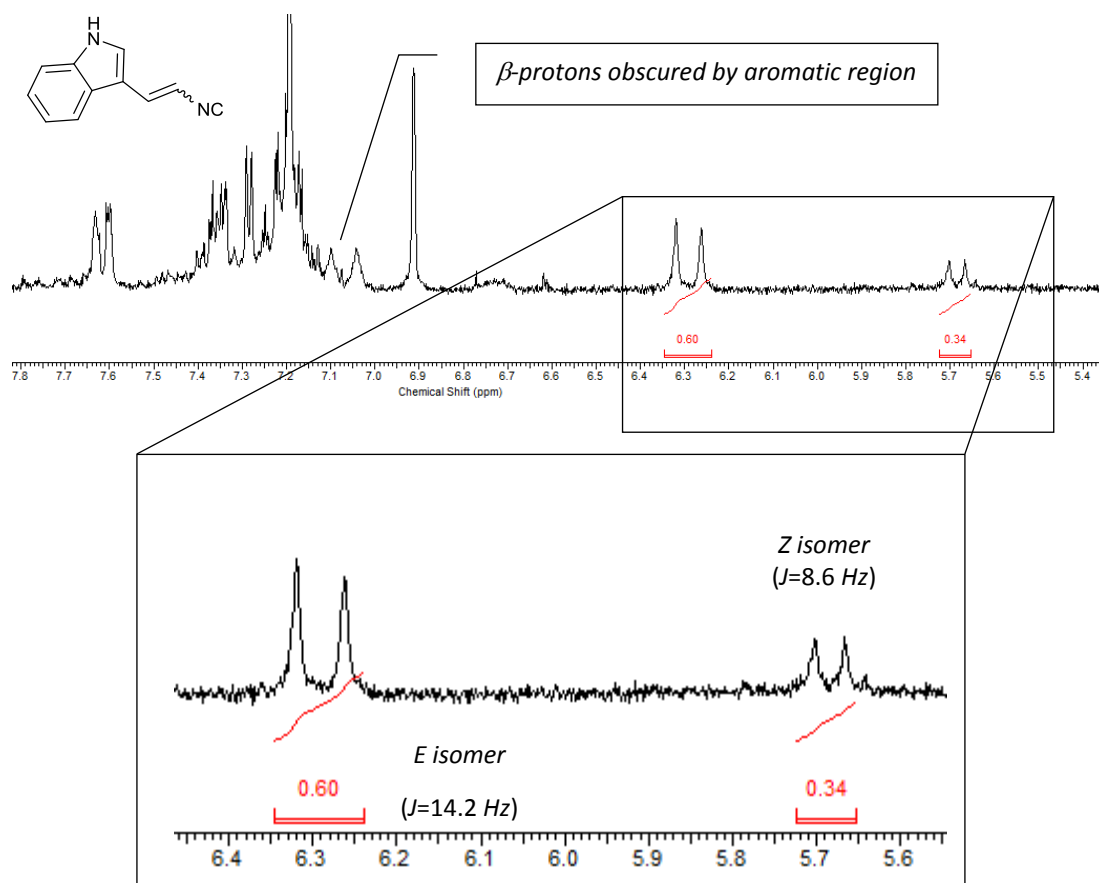


Figure 28 Crude  $^1\text{H}$  NMR spectrum of 3-indole vinyl-isocyanide **17** with alkene region expanded to show integration of *E* and *Z* isomer protons of 3 : 2 mixture of geometric isomers **17/20**.



## 2.8 Synthesis of phenyl vinyl-isocyanide **542**

Having established successful conditions that enabled PhosMIC reagent **500** to be used for the formation of the geometric isomers of 3-indole vinyl-isocyanide **17** our attention then turned to attempting to improve the *E/Z* selectivity of this reaction. Initially, we chose to investigate the *E/Z* selectivity of the HWE reaction between benzaldehyde **567** and PhosMIC **500** as substrates, with the aim of producing the *E* geometric isomer in >95 d.e. Starting with the conditions established for the synthesis of 3-indole vinyl-isocyanide **17**, PhosMIC **500** was dissolved in THF, the resulting solution cooled to -78 °C and deprotonated using 1.2 equivalents of NHMDS. After stirring for 15 minutes benzaldehyde was added to the resultant ylide, and the whole was left stirring overnight. This afforded a 3 : 2 mixture of *E/Z* isomers of phenyl-vinyl isocyanides **568** and **569**, however in this case this mixture of geometric isomers could not be separated by silica gel chromatography. Whilst there were no reports of *E*-selective HWE reactions using PhosMIC **500**, a scan of the literature revealed a number of different strategies/approaches that had been used to improve the *E*-selectivity of analogous HWE reactions of phosphonate esters (such as **570**) for the formation of  $\alpha,\beta$ -unsaturated esters (Figure 29). The majority of these reports included varying the nature of the base, solvent, and temperature used in the HWE reactions, whilst others made use of coordinating additives such as crown ethers or metal salts.

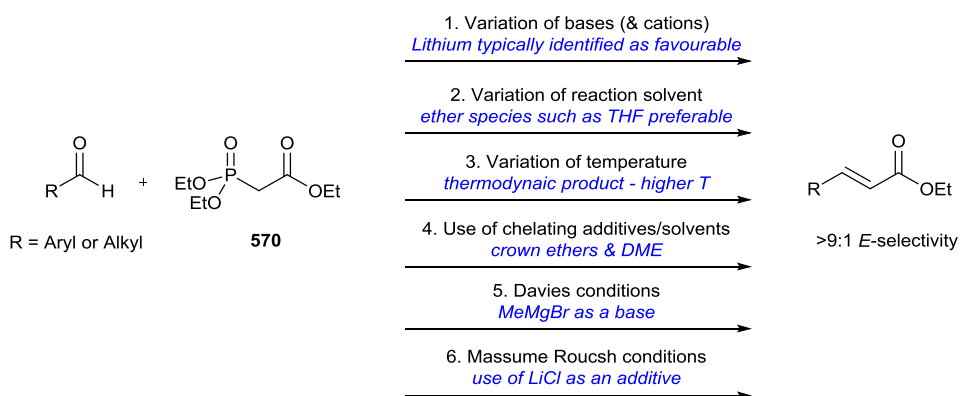
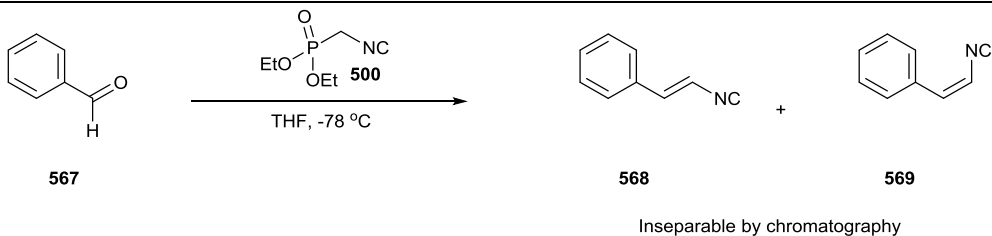


Figure 29 Literature strategies reported to enhance the *E* selectivity of HWE reactions using acetate-phosphonate **570**.

Our first optimisation screen involved changing the nature of the alkali metal base used to generate the phosphonate carbanion required for HWE homologation. The use of *n*-BuLi as a

base for deprotonation of PhosMIC **500** was unsuccessful in improving *E*-selectivity, resulting in formation of a 1 : 1 mixture of *E/Z* geometric isomers (Table 3, Entry 2). The use of freshly prepared LDA as base increased the HWE reaction's selectivity to 4 : 1 in favour of the *E*-isomer (Table 3, Entry 3), a selectivity that could not be improved upon by using one equivalent of 12-crown-4 to sequester the lithium counterion (Table 3, Entry 4). However, a major breakthrough occurred when LHMDS was used as a base for carbanion formation, giving the desired phenyl vinyl isocyanide as a 95 : 5 mixture of *E/Z* isomers in 30% isolated yield after chromatographic purification (Table 3, Entry 5). Once again, the addition of 12-crown-4 had no effect on the *E/Z* selectivity or yield of the HWE reaction when using LHMDS as a base (Table 3, Entry 6).

Table 3 Initial HWE base screen for phenyl-vinyl isocyanide **568**

				
Entry	Base	Reaction Time	12-crown-4	<i>E:Z</i>
1	NaHMDS	16 h.		3 : 2
2	BuLi	16 h.		1 : 1
3	LDA	16 h.		4 : 1
4	LDA	16 h.	Yes	4 : 1
5	<b><i>LiHMDS</i></b>	<b>2 h.</b>		<b>95 : 5</b>
6	LiHMDS	2 h.	Yes	95 : 5

Whilst we had developed conditions for the preparation of phenyl-vinyl isocyanide **568**, it soon became clear that this compound was a highly reactive and unstable molecule that was difficult to purify, handle and store. For example, neat phenyl vinyl-isocyanide was seen to polymerise when left standing on the bench for a few minutes, with complete decomposition of the product occurring during attempts to purify it by distillation. This susceptibility to polymerisation was also clearly observed when retrieving NMR samples that had been queued for analysis for an hour or more, with such samples being seen to contain increasing amounts of dark brown polymeric material over time (Figure 30). However, it was eventually discovered that samples of phenyl-vinyl isocyanide **568** could be stored at -18 °C

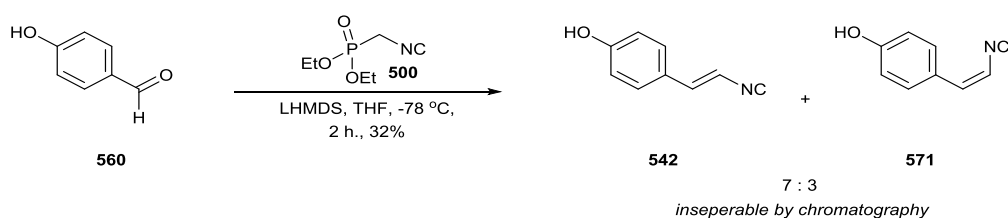
in polar solvents such as chloroform, methanol or acetonitrile for up to 14 days without significant decomposition.



Figure 30 Degrading/polymerising NMR sample containing phenyl vinyl-isocyanide **568**.

## 2.9 Synthesis of *p*-hydroxy phenyl vinyl-isocyanide **542**

Having demonstrated that PhosMIC **500** could be used to prepare phenyl vinyl-isocyanide **568** with good levels of *E* selectivity, our attention then turned to preparing the phenol vinyl-isocyanide (aglycone) **542**. The previously optimised conditions employed for the HWE reaction of benzaldehyde **567** with the lithium anion of PhosMIC **500** were modified to employ 2.5 equivalents of LHMDs, due to the presence of the acidic phenolic proton (pKa 10) of the *p*-hydroxybenzaldehyde substrate. This HWE reaction gave the desired phenol vinyl-isocyanide **542** as a disappointing 7 : 3 mixture of its *E/Z* (**571**) isomers (Scheme 66), that could not be separated by silica gel chromatography.



Scheme 66 Unselective synthesis of phenol vinyl-isocyanide **542**.

At this stage, it was considered prudent to attempt to re-optimize the HWE conditions used to prepare phenol vinyl-isocyanide **542**, and as a consequence, a range of bases were screened to try and identify more *E*-selective conditions for the synthesis of phenol vinyl-isocyanide **542**. 2.1 equivalents of a range of bases (NHMDs, LHMDs, LDA, KHMDS, DBU,

DBN) were added to a solution of PhosMIC **500** in THF (with and without crown-ether) at -78 °C, to which a solution of *p*-hydroxybenzaldehyde **560** was then added. These experiments revealed that using, DBU, DBN or KHMDS as base gave no vinyl-isocyanide product (Table 4, Entries 1-3) and that NHMDS, LDA, gave poorer *E/Z* selectivities than LHMDS (Table 4, Entries 4-6). The use of LHMDS as base was optimal, affording a 7 : 3 ratio of the desired *p*-hydroxyphenyl vinyl-isocyanide **542** in 36% isolated yield. This time the presence of 15-crown-5 actually shut down the reaction, resulting in the formation of white precipitate upon addition of the aldehyde. (Table 4, Entry 7).

Table 4 Screen of bases commonly used for HWE reactions

	<b>560</b>		<b>542</b>	<b>571</b>
Entry	Base	additive	% conversion	% selectivity
1	DBN	-	0	-
2	DBU	-	0	-
3	KHMDS	-	0	-
4	NaHMDS	-	100	66 : 33
5	LDA	-	100	55 : 45
<b>6</b>	<b><i>LiHMDS</i></b>	-	<b>100</b>	<b>70 : 30</b>
7	LiHMDS	15-Crown-5	0	-

A quick solvent screen (dichloromethane, toluene, diethyl ether) revealed that only diethyl ether was successful in affording product in a slightly inferior 66 : 34 ratio. Carrying out the HWE reaction using an inverse addition protocol, involving dropwise addition of the lithium enolate of PhosMIC **500** to aldehyde **560** in THF at -78 °C did not result in improved *E/Z* selectivity. Certain HWE reactions of phosphonate esters are known to be reversible, a property that is often used to afford their more stable *E* alkene products under thermodynamic control. Consequently, it was decided to carry out the optimised HWE reaction of the enolate of PhosMIC **500** with *p*-hydroxyphenylbenzaldehyde **560** at various reaction temperatures (-78 °C, -40 °C, 0 °C, r.t. and 65 °C) to see whether the proportion of *E*-isomer formed would increase with increasing temperature. It was found that the *E* selectivity of this HWE reaction remained essentially unchanged over this temperature

range, with lower isolated yields of vinyl isocyanide **542** being obtained at higher temperatures.

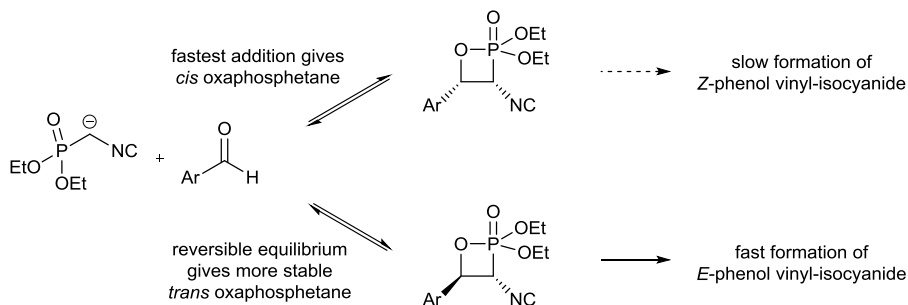
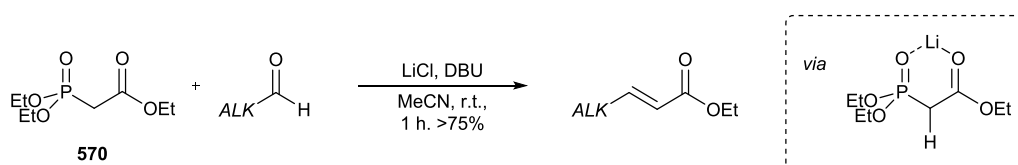


Figure 31 Mechanism of reversible HWE reactions.

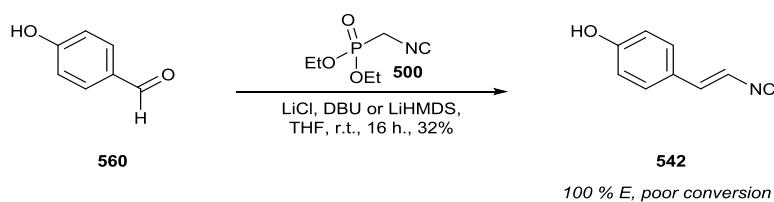
Subsequent tests on the protocol revealed that the optimum procedure in terms of selectivity/yield is to hold the reaction at -78 °C during carbanion formation and once addition of aldehyde is complete, bring the reaction rapidly to room temperature. Using this protocol phenol vinyl isocyanide was produced with 70% selectivity and isolated in 45% yield.

One reaction additive that did have an effect on the selectivity of this HWE methodology was LiCl, an effect that was originally reported by Masamune and Roush for the *E*-selective synthesis of involving triethyl phosphonoacetate (Scheme 67).<sup>184</sup> LiCl is added to the reaction so that it may coordinate to the phosphonate's  $\pi$ -bonding oxygens, a process that serves to lower the pKa of the phosphonate's methylene protons, enabling milder reaction conditions that favour the formation of reversible equilibria.



Scheme 67 Masumune-Roush protocol for vinyl esters *via* Lithium coordination to phosphonates.<sup>184</sup>

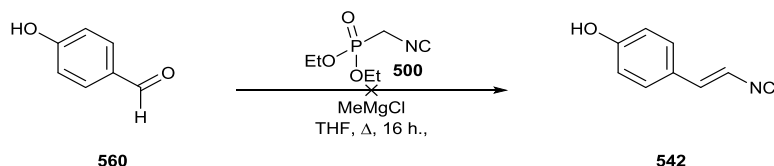
Using the conditions reported by Masumune and Roush as inspiration, LiCl was mixed with PhosMIC **500** in THF at room temperature and left to coordinate for several minutes. The complex was then deprotonated using DBU base and after stirring for 15 minutes the *p*-hydroxybenzaldehyde **560** added (Scheme 68). After 16 h. TLC analysis of the crude reaction mixture highlighted that although a new less polar unsaturated product had been formed, the starting aldehyde remained. Encouragingly, despite a low conversion (<15%) to the phenol vinyl-isocyanide **542**, analysis of the crude  $^1\text{H}$  NMR confirmed that reaction had proceeded with 100% selectivity for the *E*-isomer. Close inspection of the reaction revealed that upon addition of LHMDS a white precipitate formed, and that maybe increasing the reaction temperature would improve the lithium-phosphonate's solubility and drive the reaction to completion. Repeating the reaction at reflux and also with LHMDS base once again produced phenol vinyl-isocyanide solely as its *E*-isomer but failed to improve significantly upon the reaction's abysmal conversion.



Scheme 68 Masumune-Roush conditions applied to synthesis of phenol vinyl-isocyanide **542**.

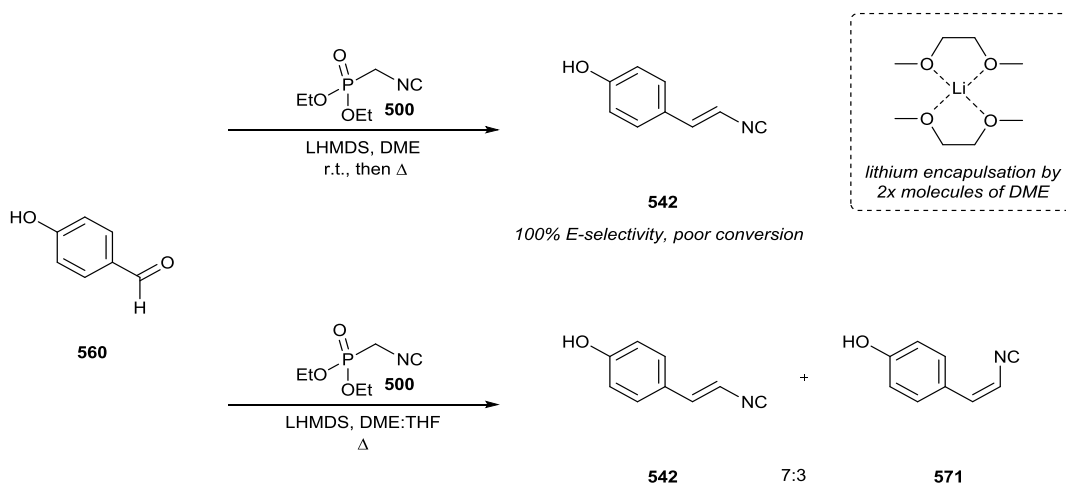
Davies *et al.* reported the use of methyl Grignard as a base for the olefination of a variety *p*-substituted benzaldehydes involving ethyl phosphonoacetate **570**, all of which had an *E*-selectivity greater than 99 : 1.<sup>185</sup> Using his conditions, PhosMIC **500** was deprotonated using methyl magnesium chloride in THF and allowed to stir at room temperature for 15 min. before *p*-hydroxybenzaldehyde was added and the whole was refluxed overnight (Scheme 69). Analysis of the crude  $^1\text{H}$  NMR identified unreacted starting materials and a lack of conversion to the desired product. It was suggested that the lack of a second oxygen upon the phosphonate species had prevented magnesium chelation, and therefore the pKa of the phosphonate's methylene protons had not been reduced, therefore preventing deprotonation by the Grignard reagent.

### Development of an *E*-selective HWE protocol



Scheme 69 Davies conditions applied to synthesis of phenol vinyl-isocyanide **542**.

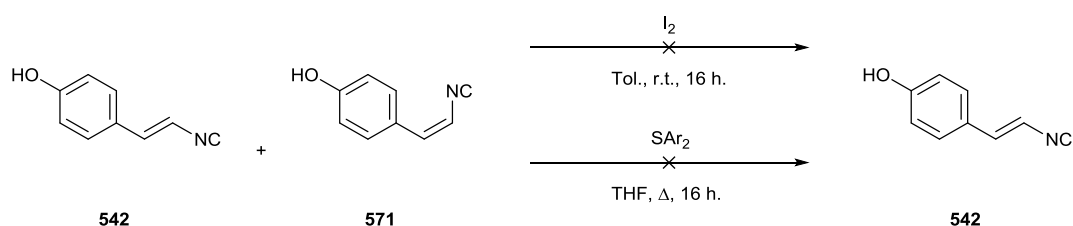
The use of 1,2-dimethoxyethane (DME) as a coordinating solvent to encapsulate the base's metal counter ions was reported by Heathcock & Thompson,<sup>186</sup> again for olefinations involving ethyl phosphonoacetate **570**. According to their protocol, the carbanion was given 15 min. at 0 °C to form using LHMDS in DME, with *p*-hydroxybenzaldehyde **560** added once the mixture had been warmed to room temperature (Scheme 70). Soon after the aldehyde's addition, precipitation occurred and upon leaving the reaction stirring for 5 h. at room temperature no significant conversion to product had been observed, therefore the reaction mixture was heated at reflux overnight. Despite an increase of temperature, the precipitate remained and the reaction only reached 5% conversion to *E*-phenol vinyl-isocyanide **542**. Refluxing a 50 : 50 mix of THF and DME to solubilise the precipitate resulted in full conversion to product, although phenol-vinyl isocyanide was formed as a 70 : 30 mixture of its *E/Z* isomers under these conditions.



Scheme 70 Trial of DME solvent for synthesis of phenol vinyl-isocyanide **542**.

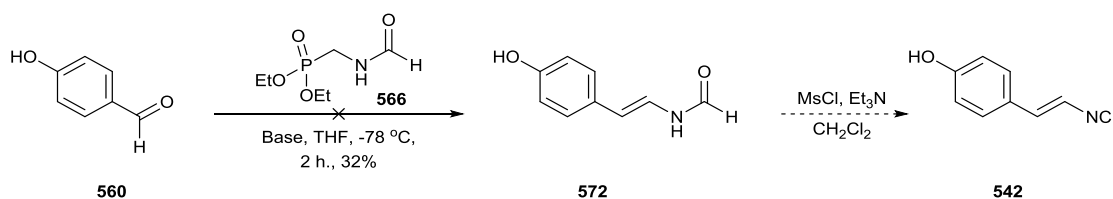
A number of examples have been reported where the reversible addition of iodine to alkene bonds has been used to isomerise mixtures of *E/Z* isomers to their thermodynamically more stable *E*-alkene isomers.<sup>187</sup> Since we were keen to eliminate the need for chromatographic

separation, we next explored the potential isomerisation of phenol vinyl-isocyanide's alkene functionality by dissolving the isomeric mixture of **542/571** in deuterated toluene and adding a small crystal of elemental iodine to the NMR tube (Scheme 71). The sample was monitored overnight by  $^1\text{H}$  NMR analysis, however no change in the ratio of the *E* and *Z* isomers was observed. Furthermore, attempts to catalyse *E/Z* isomerisation using reversible conjugate addition of diarylsulfide in THF to the alkene bonds of out vinyl-isocyanides also proved unsuccessful resulting in decomposition to afford multiple products.



Scheme 71 Attempts to isomerise a mixture of *E/Z* phenol vinyl-isocyanide **542**.

Before seeking alternative reagents/routes it was proposed that the corresponding vinyl-formamide **572** may either proceed selectively or at least provide *E/Z* isomers that would be separable by chromatography. It was then assumed that once separated the vinyl-formamide **572** could then be dehydrated using the mesyl chloride conditions developed for the synthesis of PhosMIC (Scheme 72). Unfortunately it proved impossible to deprotonate the methyl protons of formido phosphonate **566** and despite conducting a small screen of strong bases (NaH, BuLi, LDA) no phenol-vinyl isocyanide **542** could be prepared *via* HWE homologation using the formido phosphonate **572**. This is potentially why Erstabin **556** and structurally related vinyl-formamides were actually prepared *via* acid hydrolysis of their corresponding vinyl-isocyanides.<sup>188</sup>



Scheme 72 Failed HWE reaction using formido phosphonate **572**.



It should be noted that analysis of individual fractions eluted during numerous unsuccessful chromatographic attempts showed no significant change in the *E/Z* ratio of the phenol vinyl-isocyanide **542** that was recovered. Significant success in separating mixtures of *E/Z* alkenes of natural products has however been previously achieved by Mander using silica doped with silver nitrate as a stationary phase.<sup>189</sup>

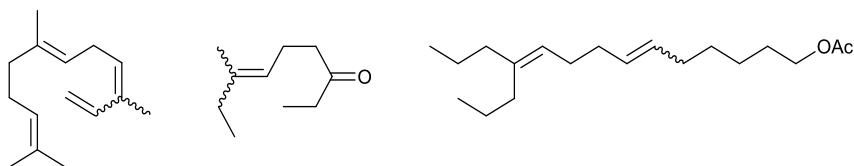
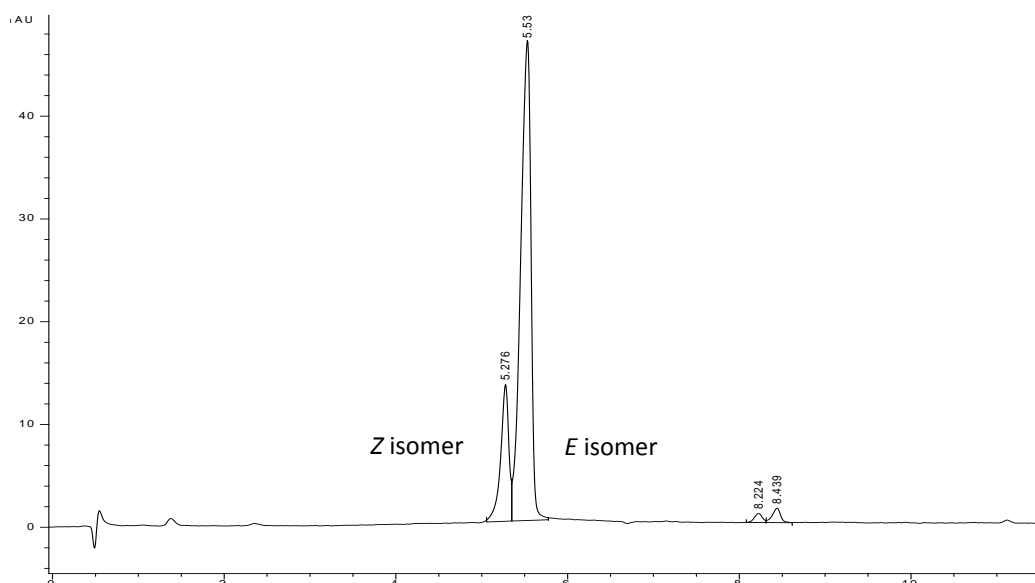


Figure 32 Examples of natural products that have had their *E/Z* isomers separated using Mander's protocol.

Consequently, silica gel TLC plates impregnated with 10% aqueous silver nitrate were prepared, and fractionation of the 3 : 2 mixture of *E/Z* isomers was attempted, however its use as a stationary phase proved unsuccessful in affording any isomer enrichment. A similar lack of success was also observed when using silver impregnated silica for flash chromatography. A closer inspection of Mander's review on using silver nitrate impregnated silica for separating mixtures of *E/Z* geometric isomers revealed that the vast majority of successful separations that have been reported involve electron-rich alkenes. Therefore, it is likely that the unsuccessful chromatographic separation of our *E/Z* isomers is a result of the presence of an electron poor alkene group that has insufficient electron-density to coordinate effectively to the silver atoms during the separation process.

As a last resort, phenol vinyl-isocyanide **542**, as a 3 : 2 mixture of its *E/Z* isomers was sent to Rob Field (John Inns Centre) for separation by reverse phase HPLC. After multiple attempts, the two isomers were separated using their analytical column (Figure 33), although the partial resolution of the two isomers highlights the difficulty in separating the phenol vinyl-isocyanide's **542** isomers.

Figure 33 UV trace for HPLC analysis of phenol vinyl-isocyanide **542**.

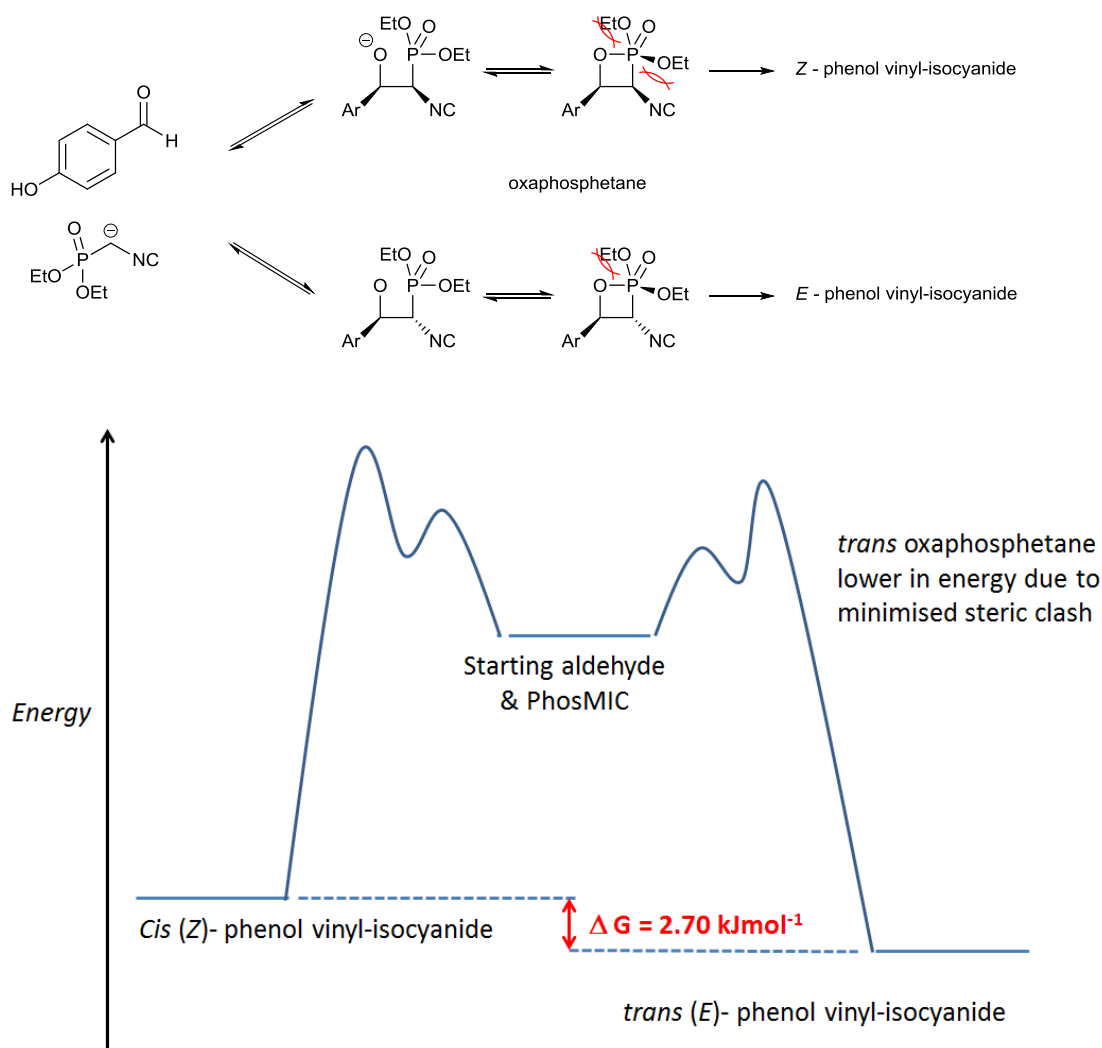
To investigate why improving the *E*-selectivity of the HWE reaction for the synthesis of phenol vinyl-isocyanide **542** had failed we decided to run some computational analysis. Using both the EDF2 and B3LYP density functionals with the 6-31G\* basis for optimisations and vibrational frequencies and the 6-311+G, we calculated the free energy for both the optimised geometries of *Z*- and *E*-phenol vinyl-isocyanide **571/542** (Table 5).

**Table 5** Computational analysis of *E* and *Z* phenol vinyl-isocyanide **542**

		EDF2		B3LYP	
		6-31G*	6-311+G**	6-31G*	6-311+G**
total energy/au	<i>cis</i>	-476.731438	-476.868500	-477.075444	-477.211560
	<i>trans</i>	-476.731669	-476.868882	-477.075894	-477.212154
$\Delta E/\text{kJ mol}^{-1}$		-0.61	-1.00	-1.18	-1.56
free energy correction/ $\text{kJ mol}^{-1}$	<i>cis</i>	272.4967	271.4035		
	<i>trans</i>	270.4026	269.3334		
$\Delta(G - E)/\text{kJ mol}^{-1}$		-2.09		-2.07	
$\Delta G/\text{kJ mol}^{-1}$		-2.70	-3.09	-3.25	-3.63
equilibrium constant for <i>cis</i> to <i>trans</i>		2.97	3.48	3.71	4.32
mole fraction of <i>trans</i> at equilibrium		0.75	0.78	0.79	0.81
<i>cis:trans</i> ratio		1 : 3	1 : 3.5	1 : 3.8	1 : 4.3

The raw energy values from all 4 data sets were tabulated in atomic units (au) and these were subsequently converted into  $\text{kJmol}^{-1}$  enabling us to conclude that the *Z*- isomer has a free energy of  $272 \pm 1 \text{ kJmol}^{-1}$ , with the thermodynamically preferable *E*-isomer having a free energy of  $270 \pm 1 \text{ kJmol}^{-1}$ . By correcting for free-energy (zero-point energy, thermal energy and entropy) it was possible to confirm the actual difference in free energy between the two isomers as being in the region of  $3 \text{ kJmol}^{-1}$ . This has been described as a negligible energy difference, and successfully evidences the challenges we are facing trying to establish conditions for a thermodynamic product that poses little to no energy preference to the kinetic product. Furthermore, by calculating the equilibrium constant for the interconversion between the *Z* and *E* forms, we can now state that at  $25^\circ\text{C}$  an equilibrium mixture contains between 75% and 81% of the more stable *trans* isomer. The ratio is particularly interesting as it essentially rationalises the crude selectivities we are observing in our HWE homologations, and to a certain extent may explain why the percentage of *Z* isomer increases during lengthy storage. By thinking about mechanism of the HWE, and its associated energy profile, it seemed appropriate to account for this energy in terms of the steric clash that arises between the phosphonate ester groups and the alkene substituents during oxaphosphetane formation. It seemed logical that the highly strained four membered ring would actively create an equilibria whereby it would reverse through the starting materials to place the alkene substituents *trans* to one another (Figure 34).

### Development of an *E*-selective HWE protocol

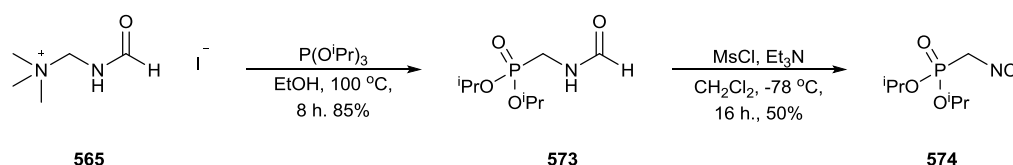


**Figure 34** Mechanism and energy level diagram for synthesis of *Z*- and *E*-phenol vinyl-isocyanide via HWE homologation.

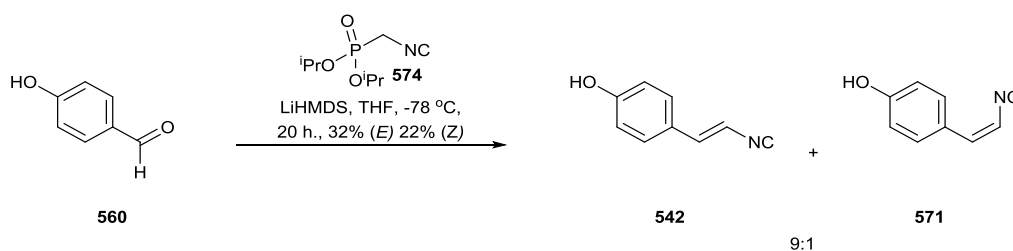
### 2.10 Development of novel, bulkier phosphonate.

From the above energy level diagram, which we theorised from the computational data, it was discussed that the use of more sterically crowded phosphonate ester might improve upon the HWE reaction's selectivity. We envisaged that by substituting the phosphonate's ethyl ether groups with larger *iso*-propyl ether groups, we would heavily disfavour the *cis*-oxaphosphetane as a result of increased steric bulk. This concept of phosphonate modification was not without precedent since Still and Gennari had developed a *bis*-trifluorinated phosphonate version of the usual triethyl phosphonoacetate,<sup>190</sup> whilst Ando has developed her own bisarylphosphonate species.<sup>191</sup> Whilst both of these novel phosphonate esters had resulted in *Z*-selective H.W.E homologations, we were not deterred

since both protocols had been for the synthesis of arylates and we remained optimistic that this selectivity would not necessarily be the case for our non-chelating isocyanide substrate. To this end, the quaternary amine salt **565** used for the synthesis of the ethyl derivative (PhosMIC **500**) was treated with triisopropyl phosphite and reacted under the previously used Arbuzov conditions to afford formamide **573**, which upon dehydration with methanesulfonyl chloride in the presence of triethylamine base yielded the novel <sup>i</sup>Pr-PhosMIC **574** (Scheme 73).

Scheme 73 Synthesis of <sup>i</sup>Pr-PhosMIC **574**.

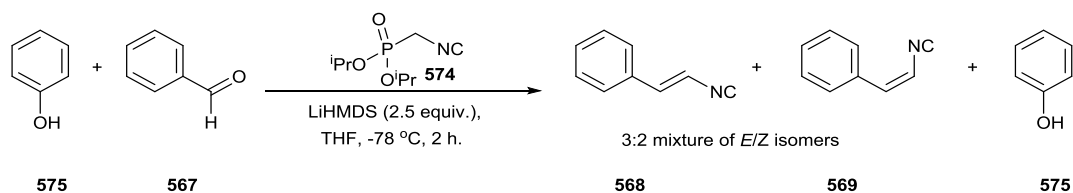
Pleasingly, when this bulkier phosphonate **574** was reacted with *p*-hydroxybenzaldehyde **560** under the optimal HWE conditions previously developed, an increase in *E* selectivity from 7 : 3 to 9 : 1 was observed (Scheme 74).

Scheme 74 Un-selective synthesis of phenol vinyl-isocyanide **542**.

## 2.11 Protection of phenol functionality.

Despite extensive work targeted towards an *E*-selective synthesis of phenol vinyl isocyanide **542** via HWE reaction with PhosMICs **500/574** and *p*-hydroxybenzaldehyde **560** having been undertaken, it appeared that the acidity of the phenol group was still preventing the development of a truly *E*-selective HWE reaction for  $\alpha,\beta$ -unsaturated isocyanides. To

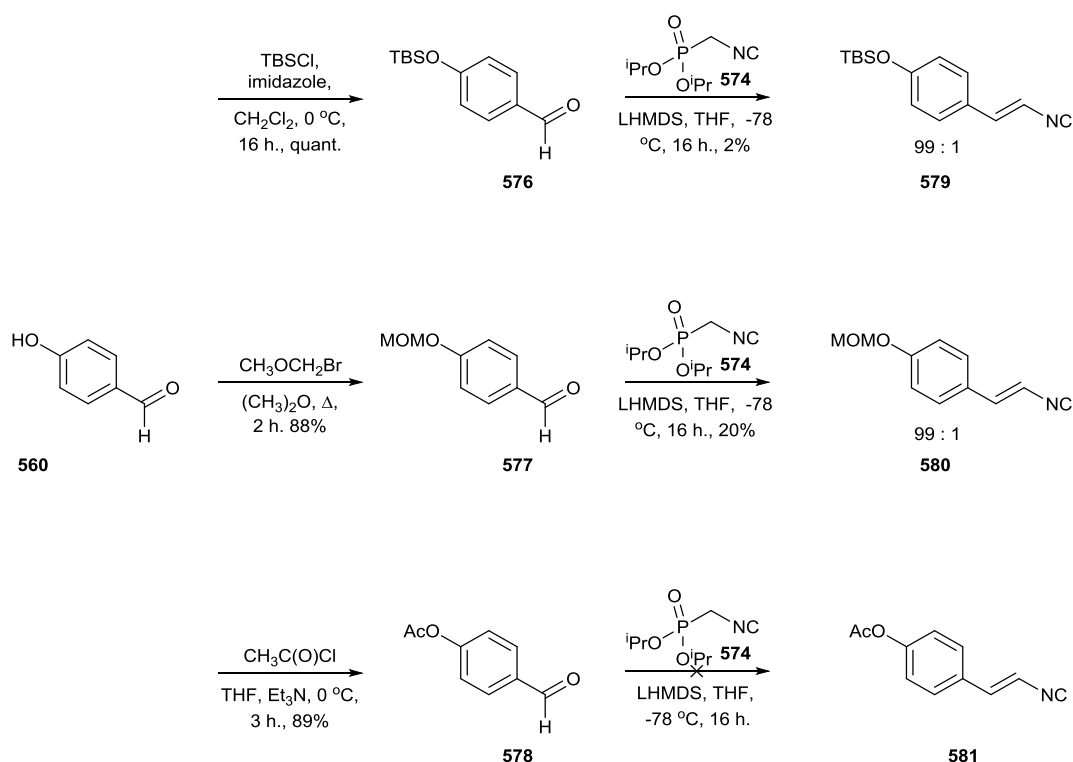
investigate the validity of this hypothesis one final preparation of phenyl vinyl-isocyanide was carried out in the presence of pure phenol **575**. To this end, a mixture of phenol and benzaldehyde was added to deprotonated <sup>i</sup>Pr-PhosMIC **574** and allowed to stir under the optimum conditions reported in Table 4 (Scheme 75). Analysis of the crude <sup>1</sup>H NMR spectrum proved to be most interesting, since the presence of phenol had indeed destroyed the selectivity of the HWE reaction, returning both isomers of phenyl vinyl isocyanide **542**.



Scheme 75 Investigation of effect of phenol functionality upon HWE selectivity.

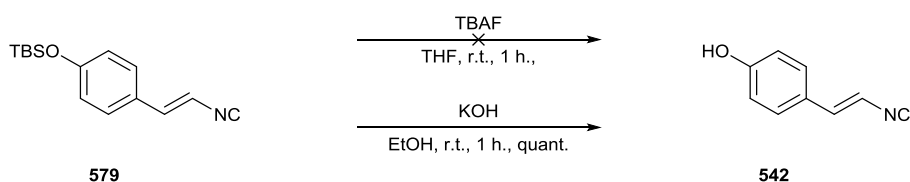
Returning to the reaction of *p*-hydroxybenzaldehyde **560** with PhosMIC **500** (c.f. Scheme 66) the effect of increasing equivalents of LiHMDS base was scrutinised by carrying out reactions that contained 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 equivalents of LiHMDS. These tests confirmed that a minimum of 2 equivalents of base were required for full conversion to phenol vinyl-isocyanide and that 2.5 equivalents were optimal. Alarming however, they also showed the inverse relationship between the equivalents of base and the selectivity of HWE reactions. To reduce the acidity of the substrate we sought to protect the phenol group as its silyl ether (TBS), methoxymethyl acetal (MOM) and acetate and then subject these aldehydes to the previously optimised HWE conditions using just 1.2 equivalents of LiHMDS base (Scheme 76). Since the  $\alpha,\beta$ -unsaturated isocyanide functionality had already proved to be highly sensitive, these protecting groups were chosen with consideration for their ability to be easily removed without the need for using strong acid. The silicon protecting group was installed by treating *p*-hydroxybenzaldehyde **560** with *tert*-butyldimethylsilylchloride and imidazole base. Initial attempts to purify the *O*-silyl benzaldehyde **576** by flash column chromatography were unsuccessful; however a kuglerhor distillation under reduced pressure proved efficient, affording *O*-silyl protected aldehyde **576** in near quantitative yield. Reflux of *p*-hydroxybenzaldehyde **560** with bromomethylmethyl ether in the presence of base afforded the *O*-MOM protected aldehyde **577**, whilst 4-formylphenyl acetate **578** was accessed by mixing with acetyl chloride in the presence of Et<sub>3</sub>N base. Pleasingly the *O*-silyl

protected substrate **579** underwent HWE homologation with 99 : 1 selectivity for the desired *E*-isomer of *O*-silyl protected vinyl-isocyanide **579**. A similar selectivity was observed when preparing *O*-MOM protected vinyl-isocyanide **580**. Use of the *O*-acetate **578** failed to produce any of the desired vinyl-isocyanide product, and this was assumed to be a result of competing ketene-type decomposition of the acetate group to produce *p*-hydroxybenzaldehyde **560**.



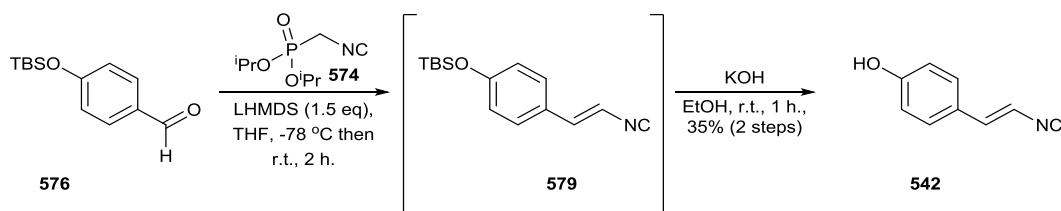
Scheme 76 Synthesis of *O*-protected benzaldehydes **576**, **577**, **578** and subsequent HWE reactions.

Having now isolated a sample of silyl-protected isocyanide **579**, generation of the target phenol vinyl-isocyanide **542** by silyl ether removal was attempted using tetra butyl ammonium fluoride (TBAF), although this was without success (Scheme 77). Under these relatively acidic conditions degradation of the  $\alpha,\beta$ -unsaturated isocyanide functionality was observed, and so milder, non-acidic conditions were sought. Pleasingly, when silyl-protected isocyanide **579** was dissolved in ethanol and treated with hydroxide, the resultant potassium ethoxide effectively deprotected the phenol to afford the target phenol vinyl-isocyanide **542** as a 95 : 5 mixture of its *E/Z* isomers (Scheme 77).

Scheme 77 Synthesis of *E*-phenol vinyl-isocyanide **542** via desilylation.

## 2.12 Final conditions and synthesis of phenol-vinyl isocyanide (aglycone).

Despite now having a selective route to phenol vinyl-isocyanide **542**, the low yield of this target aglycone from *p*-hydroxybenzaldehyde **560** was disappointing, with the HWE reaction being responsible for the greatest loss of mass during the two step sequence. Since monitoring of the HWE reaction by TLC and the subsequent analysis of the crude  $^1\text{H}$  NMR spectrum had shown full conversion of starting materials to product without any major impurities, the purification process was thought to be at fault. To this end, a route that no longer involved purification of silyl-protected isocyanide **579** was envisaged. Upon completion of the HWE reaction of aldehyde **560** with *i*Pr-PhosMIC **574**, the reaction mixture was worked-up as per protocol, THF removed *in vacuo* and the resultant oil quickly dissolved in ethanol and an excess of KOH added (Scheme 78). Within 1 h. the crude silyl-protected isocyanide **579** had been desilylated by the ethoxide and the resultant phenol vinyl-isocyanide purified by silica gel chromatography. Unfortunately, a slight loss of d.r. was observed for the isolated 'free' phenol vinyl-isocyanide, it being isolated as a 96 : 4 mixture of *trans*-/*cis*-isomers, with a sample left a room temperature slowly isomerising further over time.

Scheme 78 Final protocol for synthesis of *E*-phenol vinyl-isocyanide **542** (aglycone).



### 2.13 Conclusion

We sought to apply Schöllkopf's diethyl isocyanomethylphosphonate (PhosMIC **500**) reagent for the synthesis of phenol vinyl-isocyanide **542**, isolating this agylcone as its *E*-isomer upon silica gel chromatography. However, unlike the structurally related 3-indole vinyl-isocyanide **17**, the 3 : 2 mixture of *trans*-/*cis*-isomers that arise from HWE homologation were inseparable by chromatography, resulting in the need to develop an *E*-selective HWE protocol. Screening reagents, conditions and alternative procedures failed to increase the selectivity of the homologation reaction between PhosMIC **500** and *p*-hydroxybenzaldehyde **560**. We subsequently found that by increasing the size of the phosphonate ester groups from ethyl to *isopropyl* (PhosMIC **574**) we were able to increase the *E*-selectivity of our HWE reaction from 7 : 3 to 9 : 1, with total selectivity being achieved by *O*-silyl protecting the aldehyde's phenol group. This reduction in substrate acidity enabled us to use minimal LHMDS, and selectively access an *E*-vinyl-isocyanide intermediate that could be mildly desilylated to afford the desired phenol vinyl-isocyanide **542**. Unfortunately there was a slight isomerisation during desilylation/isolation of the agylcone, it being isolated with a 95 : 5 d.r.

The subsequent chapters of this thesis detail our biological evaluation of the agylcone and its use in the synthesis of the two rhamnopyranose natural products, Byeyankacin **544** & Rhabduscin **543**.

### 3 Biological evaluation of phenol vinyl-isocyanide

#### 3.1 Melogenesis suppression

Previously (chapter 2) we introduced the vinyl-isocyanide derived secondary metabolites produced by the insect pathogens *Photorhabdus* and *Xenorhabdus* bacteria to suppress their prey's immune response *via* inhibition of the tyrosinase oxidases responsible for melanin production (Figure 35).

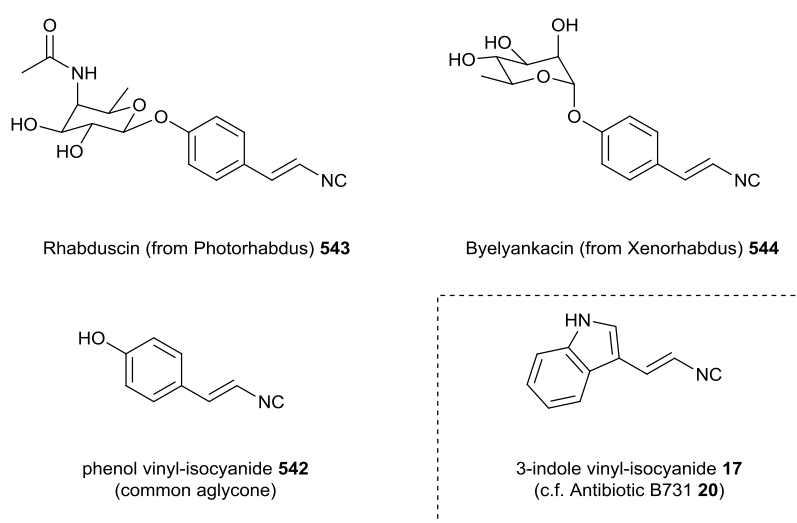


Figure 35 Structure of vinyl-isocyanide derived natural products **542**, **543** and **544**, and the structurally related 3-indole vinyl-isocyanide **17**.

Clardy has already been able to deduce the biological mode of action for these vinyl-isocyanide derived natural products,<sup>179</sup> successfully demonstrating **542**, **543** and **544**'s ability to inhibit mushroom tyrosinase and phenol oxidases (Table 1). During these studies it was noted that the free aglycone component (phenol vinyl-isocyanide **542**) of both glycosides was significantly more potent than the glycosides. This lent itself to our working hypothesis that the sugar fragment serves to deliver the free phenol vinyl-isocyanide war head to the tyrosinase active site, and consequently we sought the *E*-selective synthesis of phenol vinyl-isocyanide **542** (and 3-indole vinyl-isocyanide **17**) believing these to be the critical species for biological evaluation (chapter 2). Clardy's studies had focussed on the inhibitory activity against tyrosinase and phenol oxidase enzymes that he selected for their relation to enzymes involved in melanogenesis (Section 2.3, Scheme 61). Through collaboration with Jean van den Elsen

(Department of Biochemistry, University of Bath) we were able to use his colony of *Manduca sexta* larvae (Figure 36) to further study the effects of phenol vinyl-isocyanide **542** and 3-indole vinyl-isocyanide **17** on melanin production.

Figure 36 *Manduca sexta* (tobacco hornworm) larvae (L) and pupa (R).

During pupation, the *Manduca sexta* larvae use melanin to form their chrysalis, a dark protective shell that protects them as they mature from caterpillar to moth. Since this melanin can be collected by draining the haemolymph from the *Manduca sexta*'s tail, we selected three larvae and injected one of them with phenol vinyl-isocyanide **542** and another with 3-indole vinyl-isocyanide **17**. After feeding for one week, we studied the effects that the vinyl-isocyanides had had on melanogenesis, collecting the haemolymph of each caterpillar and exposing the pale blue solution to light. As expected the control sample (containing normal levels of melanin) instantly turned black (Figure 37). However, the samples collected from the larvae that had been dosed with the vinyl-isocyanides **542** and **17** remained clear, indicating that production of melanin by the two *Manduca sextas* had indeed been suppressed.

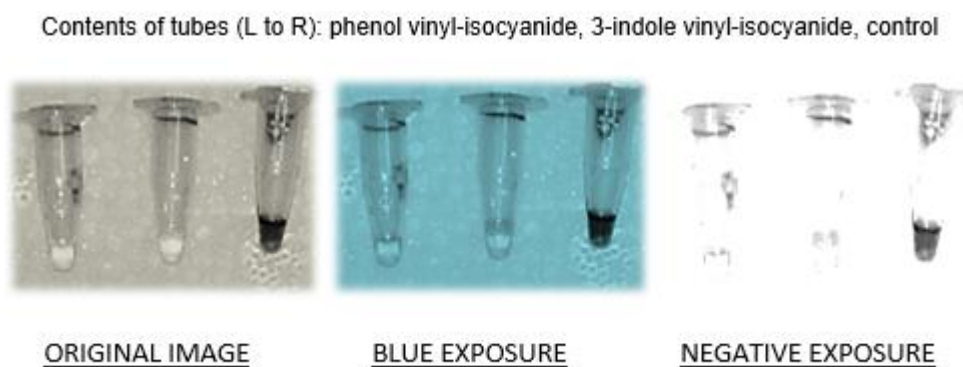


Figure 37 Vinyl-isocyanides ability to inhibit melanogenesis.

Impressively, the *Manduca sexta* survives being dosed with the vinyl-isocyanides **542** and **17**, their apparent low toxicity proving that these isocyanide metabolites serve only to suppress the immune response, enabling the nematodes to kill the host.

### 3.2 Antibiotic Activity

The fact that *Photorhabdus* and *Xenorhabdus* continue to produce the vinyl-isocyanide metabolites (Figure 35) post host death made us keen to explore the possibility of a secondary antibiotic role that potentially enables the bacteria and nematode to effectively compete against other microorganisms. To this end, we screened phenol vinyl-isocyanide **542** against three clinically relevant strains of highly resistant *S. aureus* bacteria, testing their susceptibility to **542** by disk diffusion (Figure 38). For the disk diffusion, the dispensation disk was loaded *via* soaking in a  $0.5 \text{ mg mL}^{-1}$  solution of **542** in water : methanol (95 : 5), and then incubating the inoculated petri-dishes for 16 h.

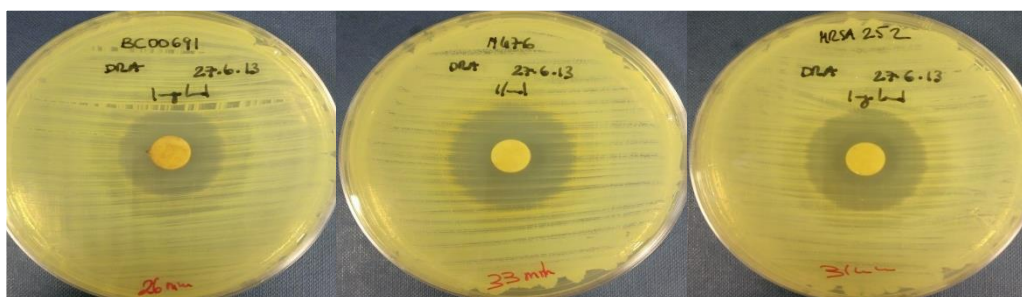


Figure 38 Disk diffusion assay for phenol vinyl-isocyanide inhibition of bacterial growth.

The clear, zone of inhibition surrounding the dispensation disk clearly demonstrates the antibiotic nature of phenol vinyl-isocyanide **542**, the three strains of bacteria all being susceptible. However, we understand that whilst disk diffusion assays provide a facile means of identifying bacterial susceptibility, the diameter of the clearance zone is dependent on a combination of the antibiotic's strength and its rate of diffusion, and therefore these assays needed to be treated as a way of identifying hit compounds. Going forward we decided to use the disk diffusion assays to identify hit compounds, quantifying their antibiotic activity against highly resistant MRSA 252 through determination of their minimum inhibitory concentrations (MIC). In the case of phenol vinyl-isocyanide **542**, the concentration required to kill 50% and 90% of the bacteria was  $49.52 \text{ } \mu\text{g mL}^{-1}$  ( $342 \text{ } \mu\text{M}$ ) and  $90.81 \text{ } \mu\text{g mL}^{-1}$  ( $626 \text{ } \mu\text{M}$ ) respectively. The results of these tests showing that the MIC values (molar) for our aglycone **542** are slightly higher than commercial antibiotic Erythromycin ( $\text{MIC}_{50} = 85.82 \text{ } \mu\text{g mL}^{-1}$  ( $117 \text{ } \mu\text{M}$ );  $\text{MIC}_{90} = 104.10 \text{ } \mu\text{g mL}^{-1}$  ( $141 \text{ } \mu\text{M}$ )) (Figure 39).

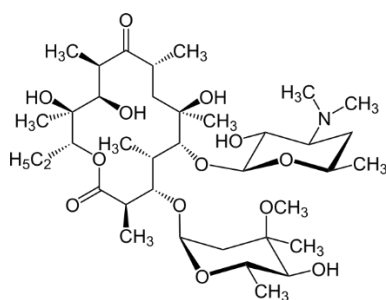


Figure 39 Structure of Erythromycin.

However, Erythromycin is a very large and difficult molecule to synthesise/isolate, a problem not shared by phenol vinyl-isocyanide **542** since it is easily prepared in 3 steps. Furthermore phenol vinyl-isocyanide's **542** small nature, water solubility and apparent low toxicity will all serve to promote this natural product as preferable drug candidate. To enable us to develop the best possible antibiotic candidate, further studies, including the synthesis of structurally related analogues was necessary. As vinyl-isocyanide antibiotics had been previously reported, we were keen to prepare these compounds for ourselves and compare their MIC<sub>50</sub> and MIC<sub>90</sub> values against MRSA 252 to those of phenol vinyl-isocyanide **542**. Initially, we determined that the MIC values for the previously synthesised 3-indole vinyl-isocyanide **17** were poorer than those for phenol vinyl-isocyanide **542** (Figure 40).

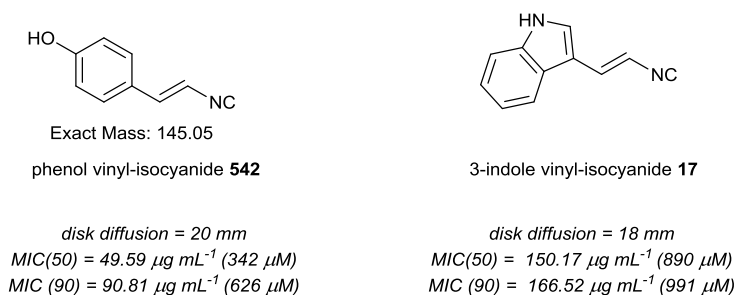
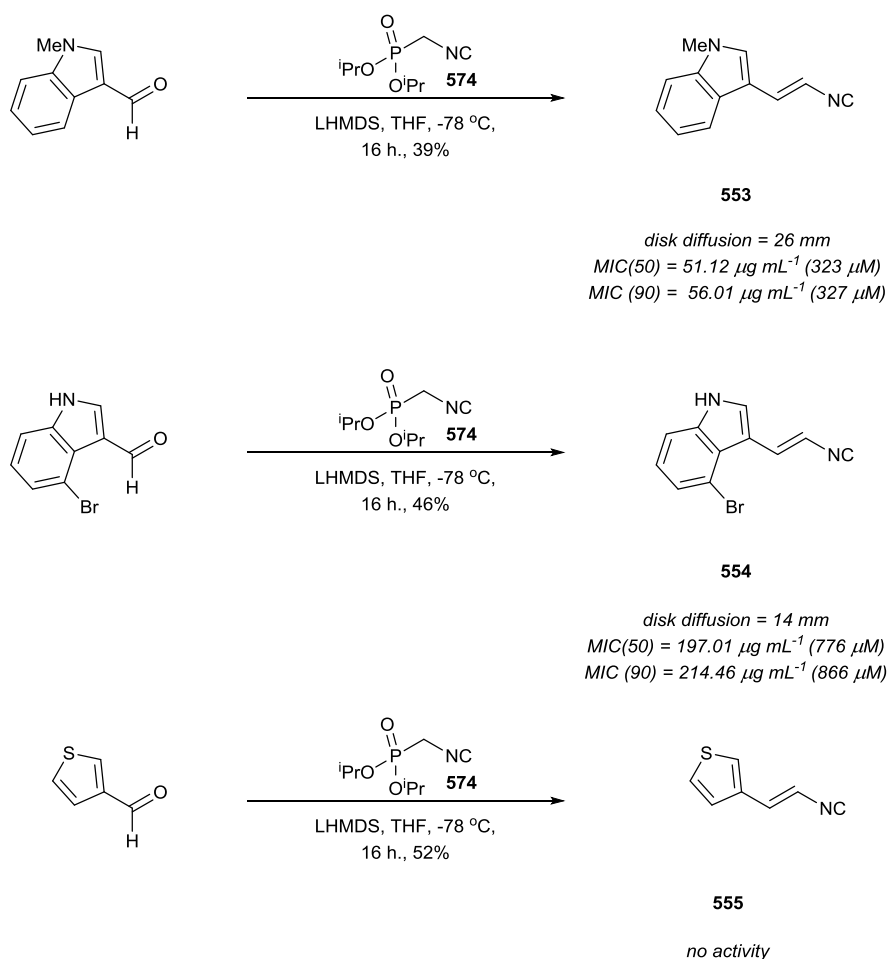


Figure 40 Antibiotic evaluation of phenol vinyl-isocyanide **542** vs 3-indole vinyl-isocyanide **17**.

Schöllkopf's SAR study had previously identified an improvement in antibiotic response when methylating the indole nitrogen of **17**, brominating the phenyl ring or switching the indole component for a thiothene unit.<sup>183</sup> Using our developed HWE protocol we synthesised the *N*-methyl **553**, 5-bromo **554** and thiothene **555** derivatives, using 2.2 equivalents of base to ensure that the phosphonate ester reagent **574** remained deprotonated (Scheme 79).

### Biological Evaluation of phenol vinyl-isocyanides



Scheme 79 Preparation and biological evaluation of previously reported 'hit' vinyl isocyanides.

The initial disk diffusion assays found that only the two indole derived species **553** and **554** inhibited bacterial growth. The methylation of the indole nitrogen (species **553**) produced an antibiotic of similar potency to phenol vinyl-isocyanide **542**, whilst bromination of the six membered ring produced a poorly antibiotic species **554**. Since *N*-methyl derivative **553** had presented itself as a serious antibiotic candidate we decide to test the susceptibility of a non-resistant strain of bacteria, MSSA 476. The indole **553** species did not fare quite so well against this new strain of bacteria its MIC<sub>50</sub> and MIC<sub>90</sub> values of 133.51 and 144.93  $\mu\text{g mL}^{-1}$  being significantly higher than the 34.19 and 100.24  $\mu\text{g mL}^{-1}$  required by phenol vinyl-isocyanide **542**. This, in our opinion, implies that the structure of phenol vinyl-isocyanide **542** would offer an antibiotic with a broader spectrum of activity, and consequently our efforts progressed into analysing the structure activity relationships (SAR) for our aglycone **542**.

### 3.3 Structure Activity Relationships

In order to practically develop an antimicrobial agent with nanomolar inhibition, we carried out a small SAR study to evaluate the significance of each of phenol vinyl-isocyanide's **542** structural functionalities. Through the design and synthesis of a series analogues, each of which would be modified to remove or replace a key aspect of the molecule, we would be able to observe the affect that each portion of the molecule has on the antibiotic response. At first, we took advantage of the previously synthesised (Scheme 77) phenyl vinyl-isocyanide **568**, with this removal of the hydroxyl group resulting in zero inhibition of bacterial growth. This was unsurprising since you have removed a polar functionality that is likely to be a key binding agent for the active site (Figure 41). We also screened two commercial, aliphatic isocyanides **582** and **583**, both of which were biologically inert, serving to further highlight the requirement of more than just an isocyanide group.

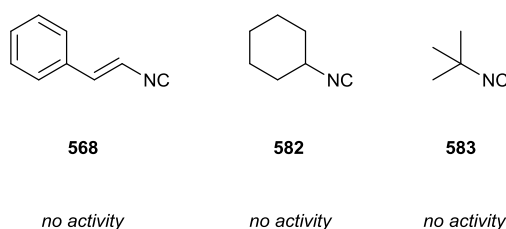
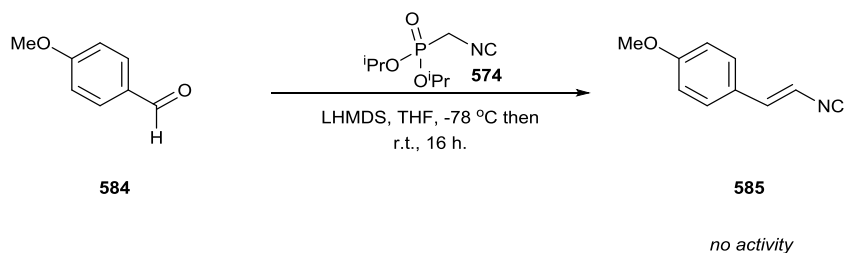


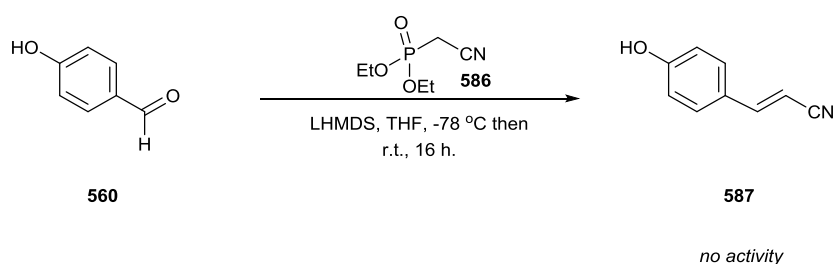
Figure 41 Biological evaluation of phenyl vinyl-isocyanide **568**, *tert*-butyl isocyanide **582**, and cyclohexyl isocyanide **583**.

Next we sought to remove phenol vinyl-isocyanide's **542** ability to act as a hydrogen bond donor through alkylation of its OH. In reality, it was simpler to synthesise *p*-(methoxy)phenyl vinyl-isocyanide **585** from *p*-methoxybenzaldehyde **584** using our HWE conditions (Scheme 80). As expected, removing the hydrogen bond donating phenol also removed the antibiotic response, there being no clearance zone surrounding **585** in the disk diffusion assay.



Scheme 80 Synthesis of methoxy(phenyl)vinyl-isocyanide **585**.

Having confirmed the importance of the phenol, and its potential to serve as a hydrogen bond donor, we decided to also validate the isocyanides' importance by synthesising the isomeric nitrile species **587**. This vinyl-nitrile was synthesised by reacting nitrile derived phosphonate ester **586** with *p*-hydroxybenzaldehyde **560** under our standard HWE conditions (Scheme 81). As expected the MRSA 252 bacteria was not susceptible to phenol vinyl-nitrile **587**, there being no clearance zone observable in the disk diffusion assay. Since the two molecules are isomeric, it would be reasonable to suggest a dependence on the isocyanide's carbanion. We would assume that binding to an active site within the bacteria, may potentially be achieved *via* a similar copper binding interaction observed for phenol-oxidase enzymes.

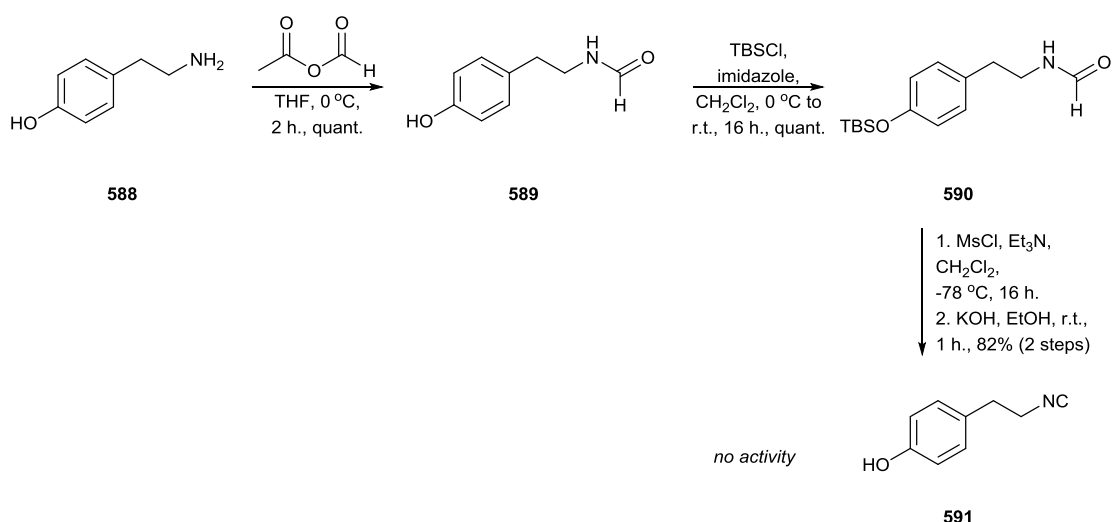


Scheme 81 Synthesis of phenol vinyl-nitrile **587**.

With nature's choice of functional groups seemingly robust, we decided to complete our SAR studies with two modifications to phenol vinyl-isocyanide's **542** core structure. First we sought to isolate the isocyanide functionality by 'saturating' the double bond and consequently de-conjugating the isocyanide. Since the isocyanide would be vulnerable to reduction, treatment of phenol vinyl-isocyanide **542** with hydride or hydrogenation was not an option. Therefore, an approach *via* dehydration of the corresponding aliphatic formamide **590** was envisaged (Scheme 82). To this end, tyramine **588** was selectively *N*-formylated using acetic formic anhydride, with the resultant phenol ethyl-*N*-formamide **589** then being *O*-silylated upon subsequent exposure to TBSCl and imidazole. With the phenol protected, it was then possible to dehydrate formamide **590** into its corresponding isocyanide *via* treatment with methanesulfonylchloride and triethylamine base, with subsequent mixing with ethoxide removing the *O*-silyl protecting groups to afford phenol ethylisocyanide **591** (Scheme 82). By isolating the isocyanide, thus preventing donation of electron density from the electron rich aromatic ring, we again observed no antibiotic inhibition in the disk diffusion assay.

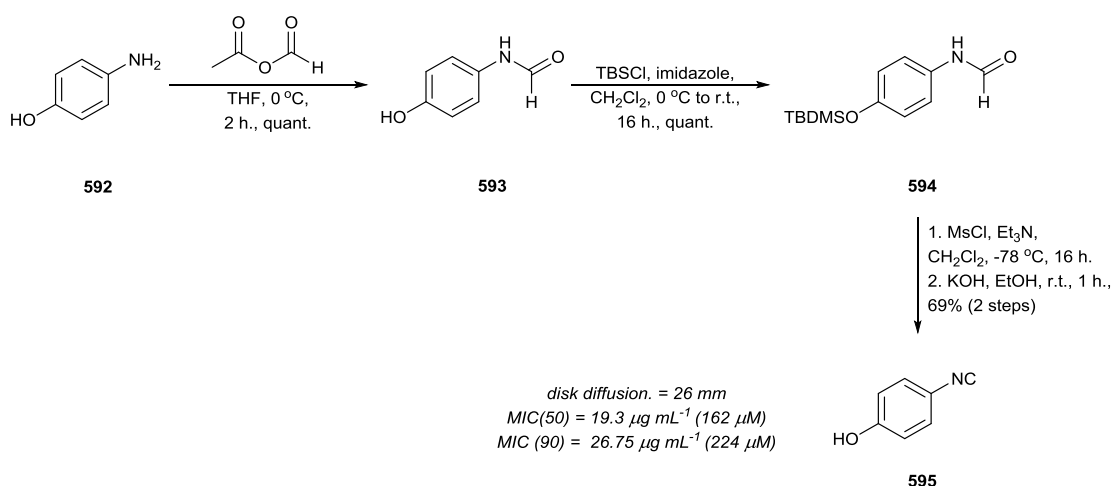


### Biological Evaluation of phenol vinyl-isocyanides



Scheme 82 Synthesis of phenol ethylisocyanide **591**.

The second alteration we wished to make to phenol vinyl-isocyanide was the removal of the ethyl linkage, therefore attaching the isocyanide functionality directly to the aromatic ring. Isocyano phenol **595** was synthesised in an analogous manner to phenol ethylisocyanide **591**, starting with the selective *N*-formylation of amino-phenol **592** (Scheme 83). Treatment of amino-phenol **592** with acetic formic anhydride resulted in formido-phenol **593**, with subsequently *O*-silylation by TBSCl and imidazole affording aryl formamide **594**. Reaction of this formamide **594** with methanesulfonylchloride and triethylamine base resulted in its dehydration to the corresponding isocyanide. 4-isocyano phenol **595** was realised upon ethoxide mediated de-silylation, although this species began to decompose quickly at r.t. Owing to the conjugated nature of the isocyanide, MRSA 252 was found to be susceptible to **595** with a 26 mm clearance diameter being observed in the disk diffusion. Interestingly isocyano phenol was very potent, having low values for both its MIC<sub>50</sub> and MIC<sub>90</sub>. Unfortunately this molecule was very unstable and difficult to handle, instantly polymerising in all solutions, and therefore deemed unsuitable for further investigation.

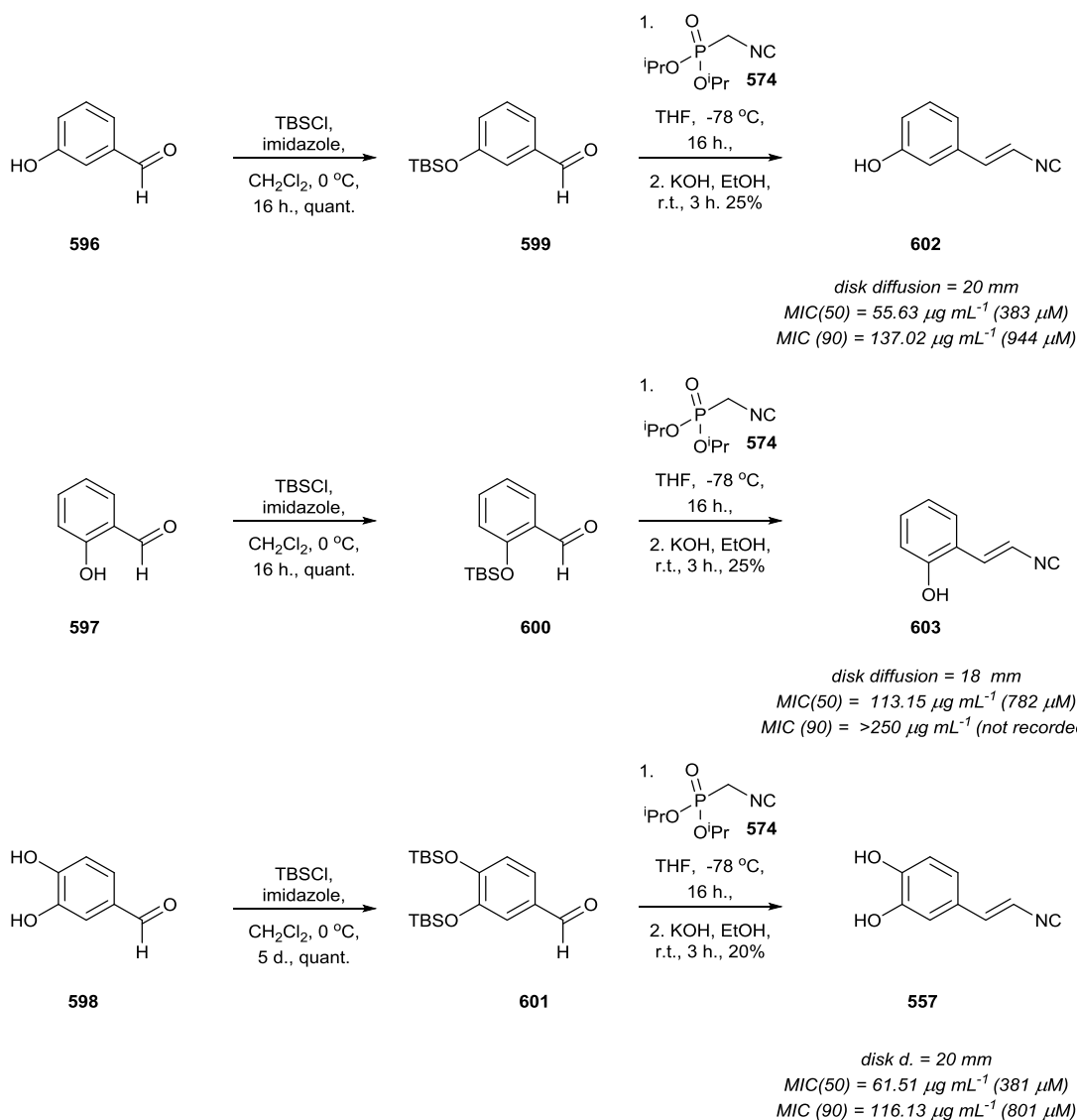


Scheme 83 Synthesis of isocyano phenol **595**.

The SAR confirmed that the natural structure produced by the *Photorhabdus* and *Xenorhabdus* bacteria is a highly effective compromise between potency and stability. In light of this, our future efforts would continue to focus around phenol derived vinyl-isocyanides.

### 3.4 Library of phenols

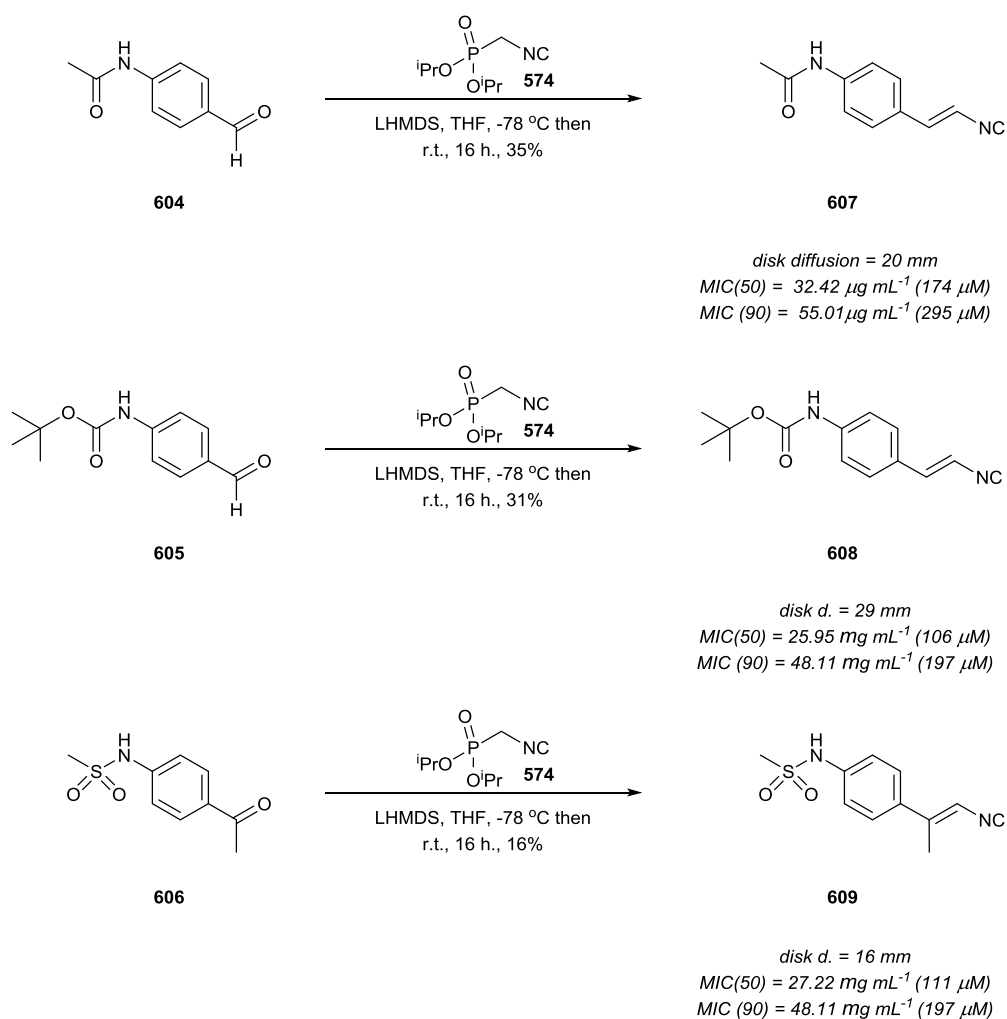
With the initial SAR results confirming the requirement of a conjugated phenol isocyanide, we decided to prepare a small library of *E*-phenolate vinyl-isocyanides and compare their biological response to the natural 4-phenol vinyl-isocyanide **542**. In order to selectively prepare the *E*-isomers of *ortho*-, *meta*- and *bis*-phenol vinyl-isocyanides **602**, **603** and **557** we began by *O*-silylating salicylaldehyde **596**, 3-hydroxybenzaldehyde **597** and 3,4-dihydroxybenzaldehyde **598**. Whilst the mono-phenol species both underwent the silylation (TBSCl/imidazole) reaction as before, the diol species required more than the standard 16 h. to reach completion by overcoming the steric crowding around the adjacent hydroxyls (Scheme 84). With all three of the silylated aldehydes **599**, **600** and **601** isolated, our attention turned to their homologation. To this end, each of the prepared aldehydes was subjected to our optimised HWE protocol, affording the three vinyl-isocyanides **602**, **603**, **557** preferentially as their *E*-isomers upon de-silylation with ethoxide. Whilst the *meta*-phenol derivative **603** and diol **557** had comparative MIC<sub>50</sub> values they did not perform as well for the MIC<sub>90</sub>, with the *ortho*-phenol **602** proving to be the poorest antibiotic out of the phenol regioisomers, potentially having a hydrogen bond donor too far away from the active sites acceptor.



Scheme 84 Synthesis of phenol-isocyanide regioisomers **602**, **603** and **557**.

### 3.5 Isostere synthesis

Although encouraged by the low toxicity demonstrated by phenol vinyl-isocyanide **542** in the model system, we were inherently aware of the pharmacokinetic and toxicological problems associated with phenol derived drugs *in vitro*. These problems arise due to phenols being readily oxidised to quinones by the human body, and we therefore synthesised three bioisostere species (Scheme 85) that would successfully mimic the physiological and electronic properties of a phenol. As usual, the vinyl-isocyanide isosteres **607**, **608** and **609** were synthesised from their parent carbonyl species **604**, **605** and **606** using our optimised HWE protocol. All three of the isosteres retained phenol-vinyl isocyanide's antibiotic response.



Scheme 85 Synthesis of bioisosteres **607**, **608** and **609**.

### 3.6 Conclusions

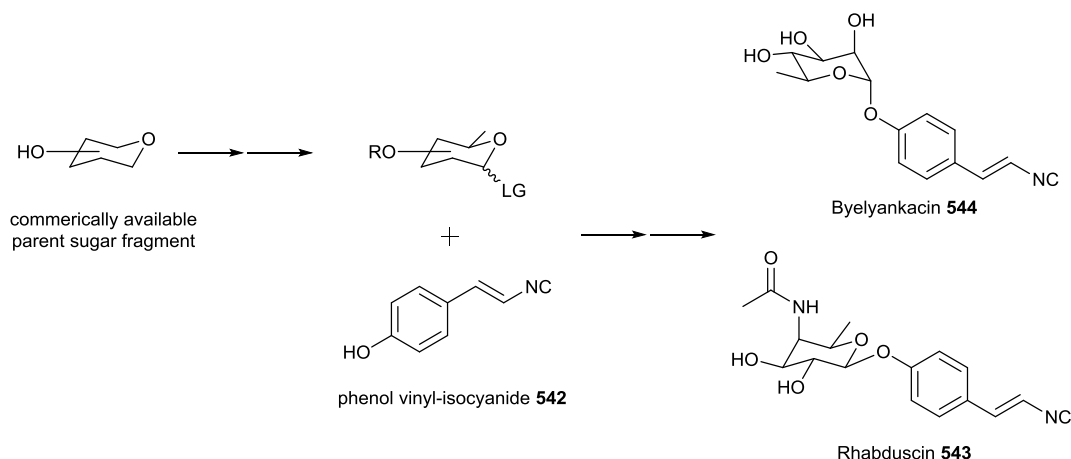
Phenol vinyl-isocyanide **542** successfully inhibited melanogenesis in *Manduca sexta* larvae, confirming the immune suppressant action of these naturally occurring vinyl-isocyanides. Subsequent biological evaluation identified these isocyanides as promising antibioitic agents, demonstrating millimolar inhibition of a highly resistant strain of *S. aureus* bacteria. Initial comparison to previously reported antibiotic vinyl-isocyanides identified phenol vinyl-isocyanide as an equal (if not superior) antibiotic candidate. The SAR studies proved the requirement of the unsaturated isocyanide functionality and regiochemistry of the natural product's phenol, which was subsequently replaced with pharmacokinetically and toxicologically preferable biosteres without reduction of antibiotic potency.

Phenol vinyl-isocyanide's **542** unique structure, low toxicity and antibiotic activity secured an additional Ph.D student (Liam Stephens) whose work to date was focussed around the synthesis of heteroaromatic vinyl-isocyanides such as **17** and **553**. He will now focus on developing a library of phenolate vinyl-isocyanides (like **542**), carrying out hit to lead optimisation to hopefully produce a vinyl-isocyanide derived antibiotic with nanomolar levels of inhibition.

## 4 Methodology for the Synthesis of Byelyankacin and Rhabduscin

### 4.1 Introduction

Previously the synthesis of  $\alpha,\beta$ -unsaturated isocyanides, *via* *E*-selective Horner-Wadsworth-Emmons homologation of their parent aldehydes was optimised (chapter 2), enabling the synthesis of *E*-phenol vinyl isocyanide **542** and a small library of antibiotic vinyl-isocyanides (chapter 3). With this in hand, our efforts now focused upon the synthesis of the two rhamnopyranoside natural products Byelyankacin **544** and Rhabduscin **543**. For this, we intended to synthesise two sugar fragments tailored for glycosylation at their anomeric position, which upon stereoselective substitution with phenol vinyl-isocyanide **542** and subsequent removal of any protecting groups would yield Byelyankacin **544** and Rhabduscin **543** as single anomers (Scheme 42).

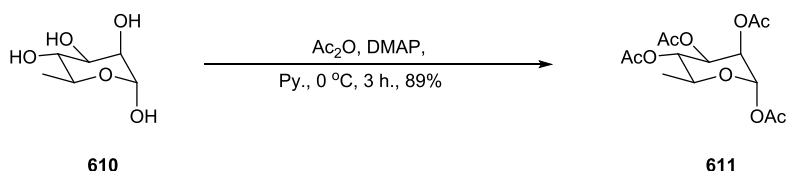


Scheme 42 Simplified representation of planned synthesis of natural products **543** & **544**.

An important consideration for these sugar syntheses would be the choice of protecting group utilised to allow for selective modification of the anomeric substituents. To this end, the hydroxyl groups of the sugars would be protected as acetates since the mildly basic conditions required for acetate removal would be well tolerated by the sensitive vinyl-isocyanide functionality of both rhamnopyranosides **543** and **544**. It was also intended to use neighbouring group participation of the C-2 acetate to control the stereochemistry of the glycosylation reactions, such that single anomers of each natural product would be formed.

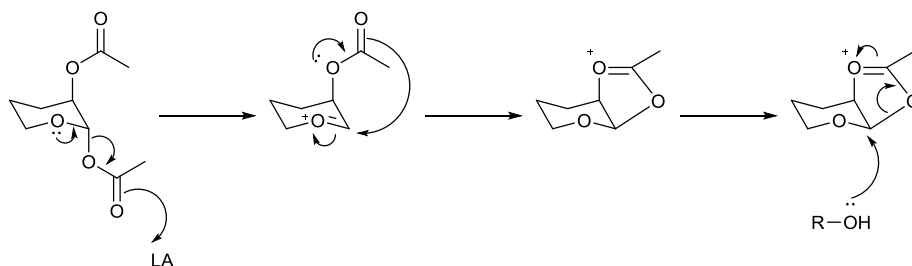
## 4.2 Synthesis of Byelyankacin

Since Byelyankacin **544** can be derived from the commercially available sugar L-Rhamnose **610**, its total synthesis would be tackled first, enabling the strategies and methodologies developed for its synthesis to be transferred onto the structurally similar, yet more complex Rhabduscin **543**. For the synthesis of Byelyankacin **544**, L-Rhamnose **610** was per-acetylated by dissolving in pyridine and treating with acetic anhydride and catalytic DMAP to afford per-acetylated Rhamnose **611** in near quantitative yield (Scheme 86).<sup>192</sup> During this reaction, the DMAP activates the acetic anhydride *via* replacement of an *O*-acetate leaving fragment with a stronger, positively charged nitrogen derived leaving group, enabling nucleophilic attack by the deprotonated sugar hydroxyl groups to proceed quickly at low temperatures.



Scheme 86 Per-acetylation of L-Rhamnose **610**.

Exploration of the literature presented a number of different glycosylation strategies for the aforementioned coupling of phenol vinyl-isocyanide **542** with a suitably derived sugar fragment. The simplest, in terms of fewest synthetic operations, appeared to be treatment of per-acetylated Rhamnose **610** with nucleophile **542** in the presence of a Lewis acid. The Lewis acid (LA) would effectively activate the anomeric acetate group through coordination to the carbonyl, weakening the anomeric C-O sigma bond. The ring oxygen's lone pair is then able to kick in and eliminate the anomeric acetate group, affording a sugar species containing an electrophilic carbonyl (Scheme 87). The neighbouring acetate group subsequently attacks this electrophilic carbonyl to form a furan-like ring that (in this case) effectively shields the anomeric carbon's  $\beta$ -face, forcing the incoming nucleophile to approach exclusively from the  $\alpha$ -face.



Scheme 87 Simplified representation of the mechanism involved with using a Lewis acid and neighbouring group participation effect to an  $\alpha$ -glycosidic linkage.

The selectivity of these reactions would be easily determined through analysis of their  $^1\text{H}$  NMR spectra. Anomers are essentially diastereomers of one another and all protons would therefore experience different chemical environments depending on the orientation of the anomeric substituent. Ratios were easily determined through measuring the integrals of the two resultant peaks for the anomeric proton, with assignment of  $\alpha$  versus  $\beta$  arising from investigation of the anomeric proton's coupling constant (Figure 43).

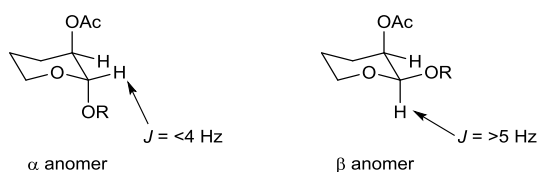
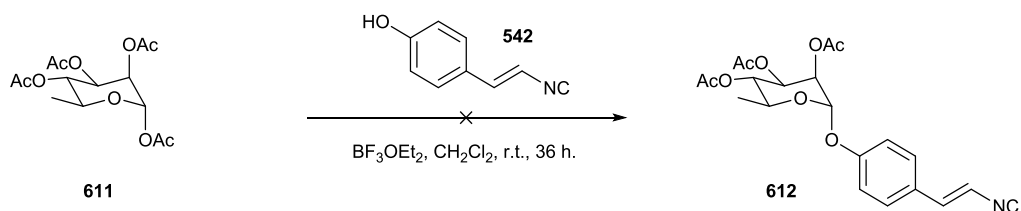


Figure 43 NMR determination of  $\alpha$  or  $\beta$  anomers based on approximate coupling constants.

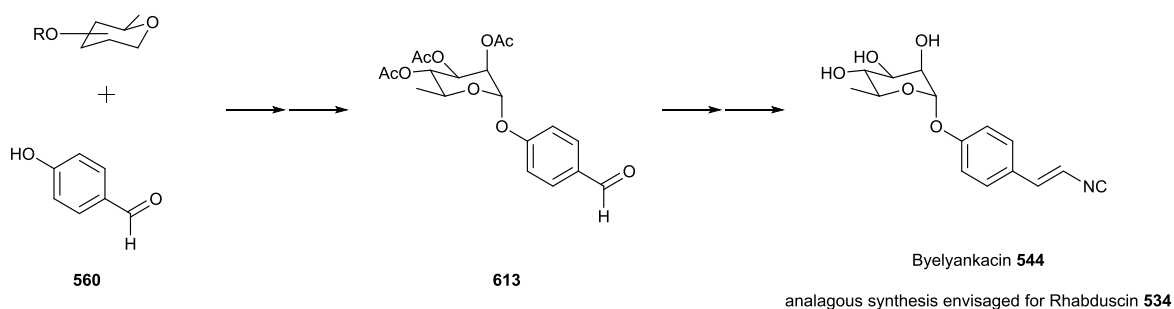
According to the procedure described by Janetka *et al.*<sup>193</sup> boron trifluoride diethyl etherate was added to a solution of per-acetylated Rhamnose **611** and phenol vinyl-isocyanide **542** in ice cold  $\text{CH}_2\text{Cl}_2$  and left to stir for 36 h. at 45 °C (Scheme 88). Monitoring of the reaction indicated full consumption of starting materials after the allotted reaction time, and the formation of several new product spots. Analysis of the crude  $^1\text{H}$  NMR spectrum confirmed consumption of the starting material (loss of anomeric acetate peak), but also indicated destruction of the phenol vinyl-isocyanide peak (loss of alkene protons) without any clear product being identifiable amongst the many random peaks present in the spectrum. It was deemed likely that the Lewis acidic conditions required to activate the sugar was responsible for destruction of the vinyl-isocyanide, therefore preventing synthesis of the desired glycosyl vinyl-isocyanide **612**.





Scheme 88 Failed coupling between phenol vinyl-isocyanide **542** and peracetate **611**.

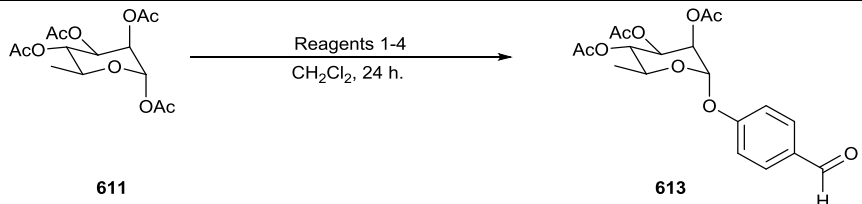
Since it appeared that the glycosylation conditions developed in the literature were not mild enough to be utilised with acid sensitive functionalities such as isocyanides, we decided to optimise conditions for the synthesis of the more stable glycosidic benzaldehyde fragment **612**, which would then undergo HWE homologation and acetate deprotection to afford Byelyankacin **544** (Scheme 89).



Scheme 89 Proposed route to Byelyankacin **544** via aldehyde **613**.

To this end boron trifluoride diethyl etherate was added to a solution of per-acetylated Rhamnose **611** and *p*-hydroxybenzaldehyde **560** and left to stir for 36 h. at 45 °C. Under these conditions glycosylation proceeded without  $\alpha$ -selectivity, with subsequent flash column chromatography returning the mixture of anomers in very poor yield. In an attempt to rectify these problems with yield and selectivity, a brief screen of Lewis acids and conditions was undertaken (Table 6 entries 1-4). Changing the Lewis acid to TMS-triflate actually resulted in reduction of the  $\alpha$ -selectivity, whilst utilisation of aluminium trichloride also failed to increase selectivity and reduced the rate of reaction such that full consumption of starting material failed to occur within 36 h. Lastly, reducing the reaction temperature failed to achieve any conversion to the desired glycosyl aldehyde **613** (Table 6, entry 4), and it was decided to seek an alternative approach for this synthesis.

Table 6 Screening of Lewis Acids

					
<div style="display: flex; justify-content: space-between; align-items: center;"> <div> <b>Table X</b>  <b>611</b> </div> <div> <b>613</b> </div> </div>					
Entry	Lewis Acid	Temperature (° C)	a : b	Conversion (%)	Yield (%)
1	BF <sub>3</sub> .OEt <sub>2</sub>	45	9 : 1	100	10
2	TMSOTf	45	8 : 1	100	12
3	AlCl <sub>3</sub>	45	8 : 1	60	15
4	BF <sub>3</sub> .Oet <sub>2</sub>	0	-	0	0
5	BF <sub>3</sub> .OEt <sub>2</sub>	r.t.	-	0	0

An alternative approach for glycosylation is to use a powerful trichloroacetimidate leaving group to activate the anomeric position towards nucleophilic attack. The reaction is driven to completion by the formation of an energetically favourable amide-like product, with neighbouring group participation once again directing the approach of the incoming phenol from the bottom face (Figure 44).

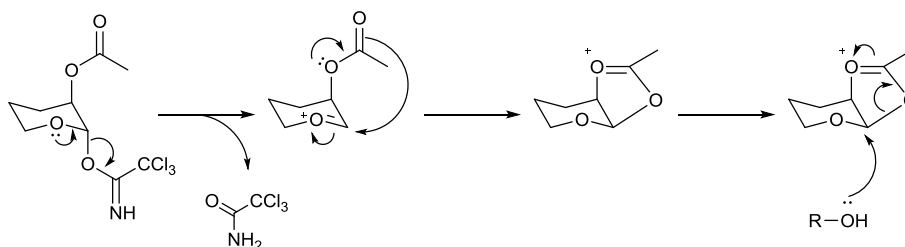
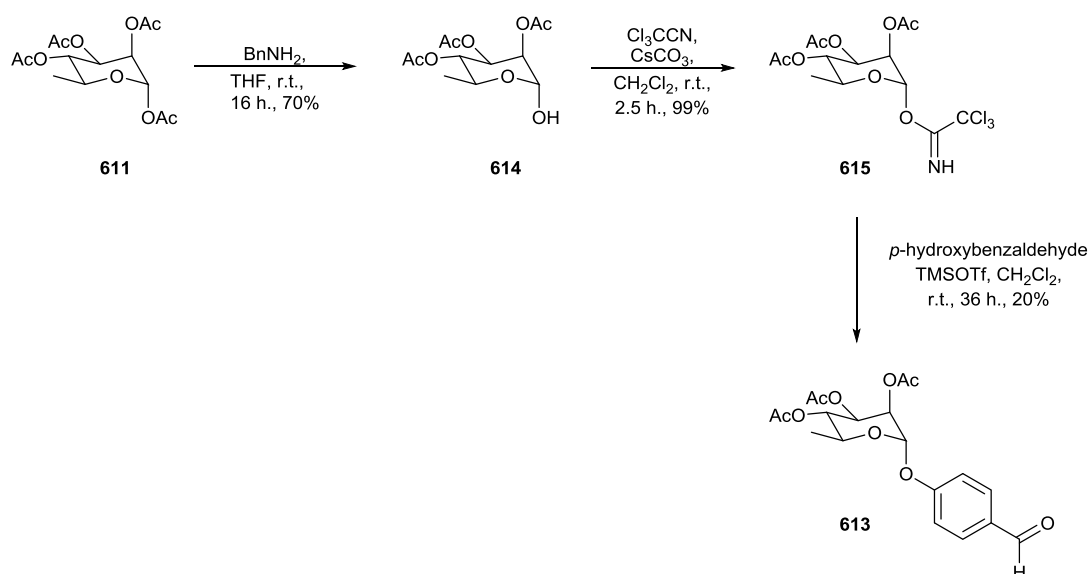


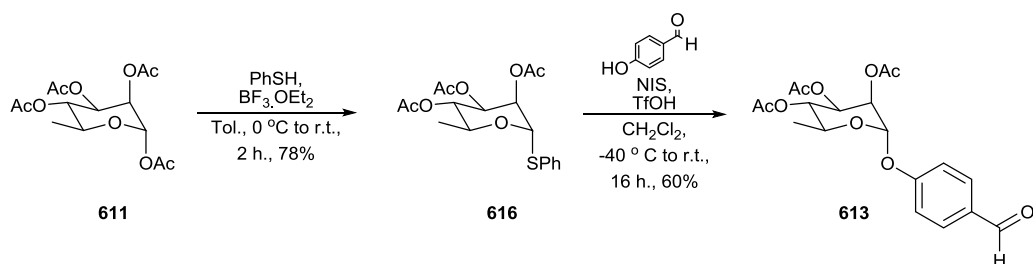
Figure 44 Mechanism for the use of an activated trichloroacetimidate sugar fragment.

For trichloroacetimidate synthesis, per-acetylated Rhamnose **611** was first selectively deprotected at its anomeric centre through reaction with benzylamine, followed by an acidic work-up to afford  $\alpha$ -hydroxy tris-acetyl Rhamnose **614** (Scheme 90).<sup>192</sup> Alcohol **614** was then reacted with trichloroacetonitrile, using caesium carbonate base, to yield trichloroacetimidate **615** that upon filtering through a short bed of silica gel was immediately reacted with *p*-hydroxybenzaldehyde **560** in the presence of TMS-triflate, affording Rhamnopyranoside **613** in 20% yield. Analysis of the <sup>1</sup>H NMR spectrum for **613** found there to be only one set of peaks for all environments, suggesting a single anomer, with confident assignment as the  $\alpha$  product owing to the anomeric proton at 5.55 ppm having the low coupling constant of 1.8 Hz.



Scheme 90 Glycosylation using trichloroacetimidate leaving group methodology.

Despite having achieved the desired stereoselective synthesis of **613**, the low yield of 20% was less than desirable, and therefore the alternative use of a thioether leaving group was explored. According to procedures outlined in the literature,<sup>194,195</sup> thioether **616** was accessed as a mixture of anomers *via* treatment of per-acetylated Rhamnose **611** with thiophenol and boron trifluoride diethyl etherate in ice cold toluene (Scheme 91). The anomeric mixture of thioethers **616** then underwent stereoselective substitution with *p*-hydroxybenzaldehyde **560** when reacted with *N*-iodosuccinamide (NIS) and catalytic triflic acid, affording the desired rhamnopyranose **613** in 60% yield.



Scheme 91 Glycosylation using thioether leaving group methodology.

In this reaction, it is believed that the electrophilic iodine (from NIS) coordinates to the sulphur atom, activating the thiophenol leaving group to enable facile nucleophilic attack from the phenol oxygen *via* a similar mechanism to that of the Lewis acid activation strategy (Figure 45).

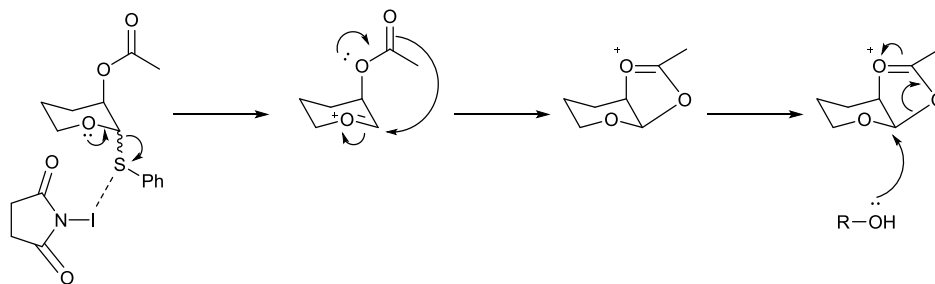
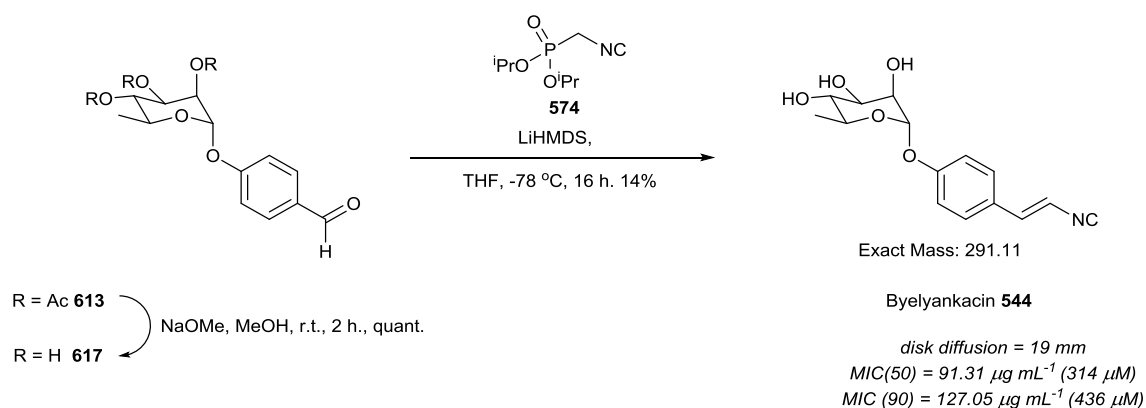


Figure 45 Mechanism of NIS mediated activation of thioethers and subsequent glycosylation.

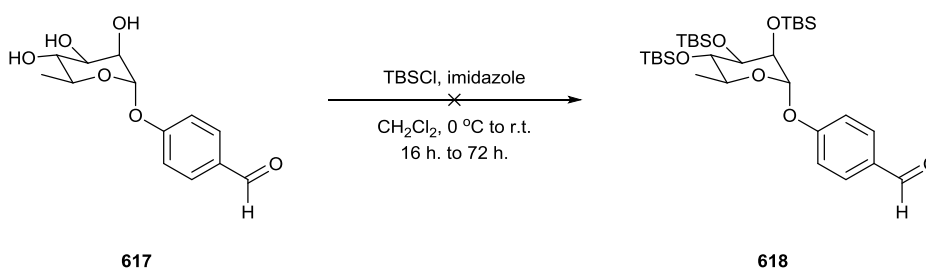
Having now achieved a highly selective method for synthesising glycosyl benzaldehyde **613**, our attention turned to generating the  $\alpha,\beta$ -unsaturated isocyanide functionality of Byelyankacin **544** *via* HWE homologation. Having previously encountered acetate instability during HWE homologation (Chapter 2), glycosyl aldehyde **613** was first treated with sodium methoxide in methanol to remove all of the acetate protecting groups, affording *tris*-hydroxy-aldehyde **617** in near quantitative yield (Scheme 92). Reaction of **617** with *i*Pr-PhosMIC **574** under the optimised HWE conditions and 4.5 equivalents of LHMDS base resulted in complete conversion to Byelyankacin **544**. The natural product was isolated by chromatography as 93 : 7 mixture of *cis*-/*trans*- isomers, and its NMR spectra found to be consistent to that report by Nakagawa and co-workers.<sup>196</sup> Byelyankacin was subsequently screened for its antibiotic activity and found to have similar MIC<sub>50</sub> value to its aglycone (phenol vinyl-isocyanide) unit, possibly confirming our hypothesis about the sugar residue's presence as a transport vector.



Scheme 92 Completed synthesis of byelyankacin **544**.

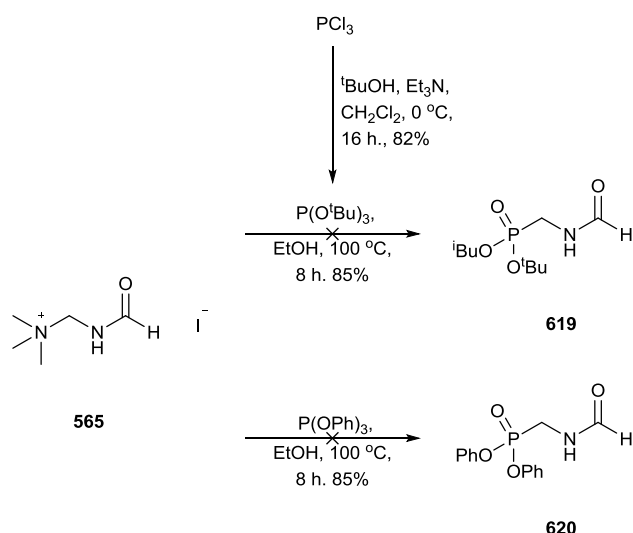
### 4.3 Attempts to fix *E* selectivity for sugar natural products

Despite the extensive optimisation work reported in chapter 2, the presence of additional hydroxyl groups upon glycosidic aldehyde **617** resulted in a slight reduction in *E*-selectivity during the HWE reaction responsible for the synthesis of Byelyankacin **544**. Although ambitious, global *O*-silylation of Byelyankacin's parent glycosidic aldehyde **617** was attempted using 5 equivalents of *tert*-butyl dimethyl silyl chloride and a large excess of imidazole base (Scheme 93). Analysis of this reaction by TLC and  $^1\text{H}$  NMR revealed a complex mixture of products, which were assumed to be a mixture of unreacted starting materials, plus the various regioisomers associated with a mixture of the various *mono/bis/tris* silylated products. Despite the use of highly forcing conditions, no further progress towards *tris*-silyl rhamnopyranoside **618** was made.



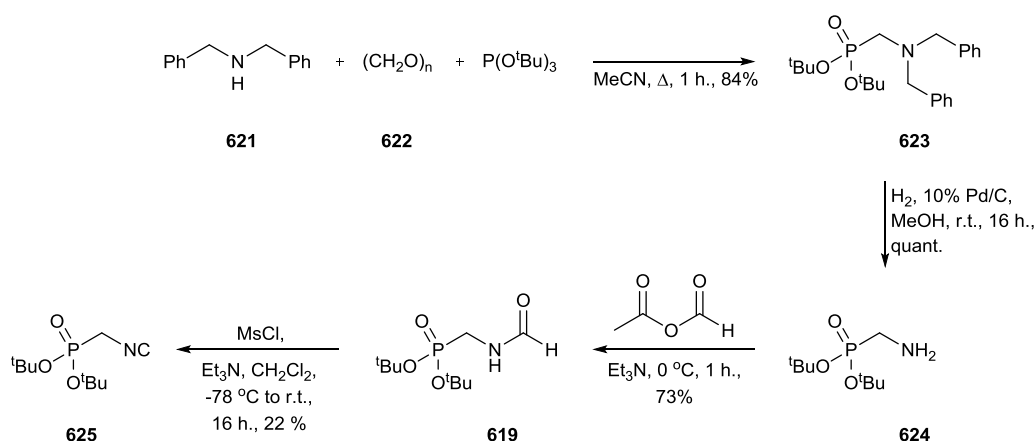
Scheme 93 Attempted tris-silylation of sugar species.339

Leaving this ill-fated global silylation behind, we envisioned that we could build upon our successful improvement in *E*-selectivity using  $^i\text{Pr}$ -PhosMIC **574** by designing and synthesising bulkier phosphonates substituted with either *tert*-butyl ethers or phenyl ethers. Upon accessing tri-*tert*-butyl phosphite *via* substitution of phosphorus trichloride with *tert*-butanol (Scheme 94), both the tri-*tert*-butyl and triphenyl phosphites were reacted with the usual amine salt **565** under Arbuzov conditions. However, the refluxing of tri-*tert*-butyl phosphite and triphenyl phosphite with the quaternary amine salt under Arbuzov conditions failed to afford either of the expected products (**619** or **620**) (Scheme 94). It appeared that the bulky nature of these two phosphites had rendered them unable to approach/substitute at the carbon centre adjacent the larger tertiary amine leaving group.



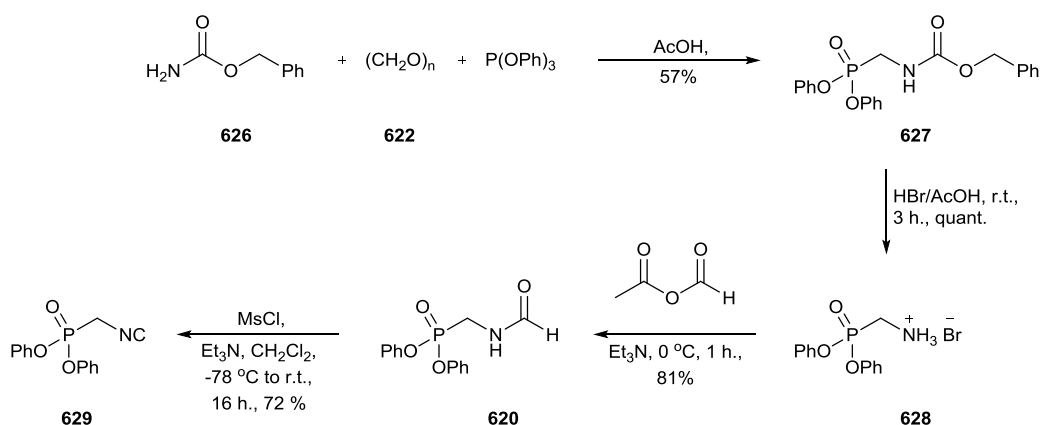
Scheme 94 Failed Arbuzov reaction with larger trialkyl phosphites.

To overcome the steric limitations of the Arbuzov reliant protocol developed for the synthesis of the original two phosphonates **566** and **573**, new routes to each of the required formamide precursors were developed. For the synthesis of  $t\text{Bu}$ -PhosMIC **625** (Scheme 95), dibenzylamine **621** was mixed with  $p$ -formaldehyde **622** and the resulting slurry treated with the previously synthesised tri-*tert*-butyl phosphite. Refluxing this mixture provided access to the desired phosphonate **623**. Hydrogenation of this dibenzyl protected amino-phosphonate **623** yielded amine **624** that was formylated upon treatment with acetic formic anhydride, generating phosphonate formamide **619** that in turn was dehydrated under the standard methanesulfonylchloride/triethylamine protocol to afford the desired  $t\text{Bu}$ -PhosMIC **625**.



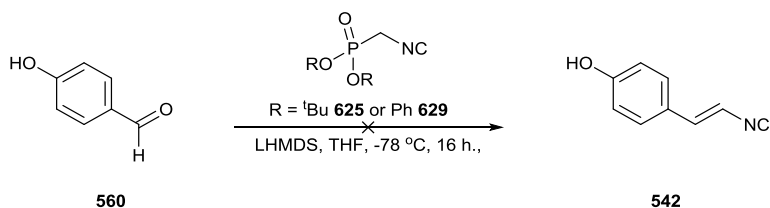
Scheme 95 Synthesis of  $t\text{Bu}$ -PhosMIC **625**.

Meanwhile, for the synthesis of Ph-PhosMIC **629** (Scheme 96), a subtly different approach developed by Sieńczyk and co-workers was employed.<sup>197</sup> To this end, benzyl carbamate **626** was mixed with *p*-formaldehyde **622** and triphenyl phosphite added to afford phosphonate-carbamate **627** that was subsequently converted to the corresponding amine salt **628** upon exposure to a solution of hydrogen bromide in acetic acid. The target Ph-PhosMIC **629** was achieved upon free-basing amine salt **628** using triethylamine and *in situ* formylation of the resultant amine using acetic formic anhydride, the generated formamide **620** undergoing dehydration using methanesulfonyl chloride to afford Ph-PhosMIC **629**.



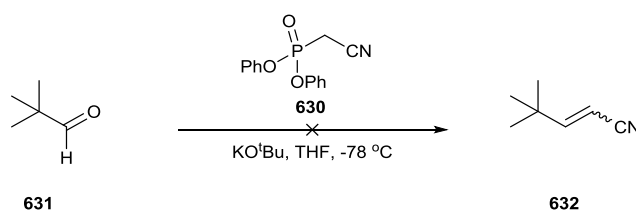
**Scheme 96** Synthesis of Ph-PhosMIC 629.

To investigate whether further increasing the steric bulk of the phosphonate ester would serve to improve *E*-selectivity in the HWE reaction, both of these novel PhosMICs (<sup>t</sup>Bu **625** and Ph **629**) were reacted with *p*-hydroxybenzaldehyde **560** under the optimal HWE conditions previously developed. For this test, each of the two phosphonates **625** and **629** were dissolved in anhydrous THF, cooled to -78 °C and deprotonated by LHMDS base. After stirring for 15 minutes, *p*-hydroxybenzaldehyde **560** was added, the solution warmed to r.t. and the whole left stirring under nitrogen for 16 h. (Scheme 97).



Scheme 97 Failed HWE reaction using novel bulkier phosphonates.

It was highly disappointing that neither of these bulkier phosphonate reagents (**625** and **629**) underwent the desired HWE reaction intended to access phenol vinyl-isocyanide **542** with excellent levels of *E*-selectivity. Analysis of the crude  $^1\text{H}$  NMR of both reactions revealed a fairly clean mixture of the starting benzaldehyde **560** and phosphonate **625** or **629**. Initially we were concerned that these phosphonate esters were too bulky to undergo deprotonation, and therefore a lack of carbanion formation had caused the reactions to fail. However, the nitrile version **630** of Ph-PhosMIC **630** had been shown to participate in HWE reactions using potassium *tert*-butoxide as a base (Scheme 98).<sup>198</sup>



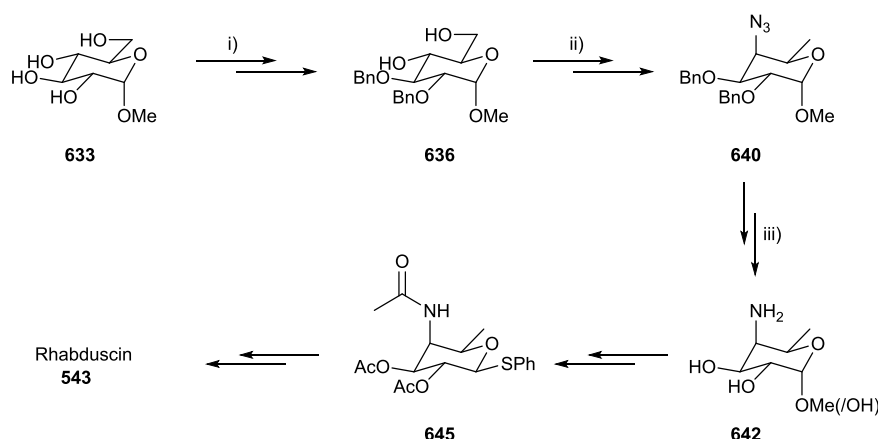
Scheme 98 Zhang's HWE homologation using diphenyl cyanophosphonate **630**.<sup>198</sup>

Given this precedent, we repeated the above test reactions (Scheme 97Scheme 94) using potassium *tert*-butoxide in place of the LHMDS. Unfortunately this modification was not successful, with the crude  $^1\text{H}$  NMR spectra of these reactions again being consistent with those of the unreacted starting materials. Regrettably given the time limitations at this stage of the Ph.D, and the acquirement of a semi-preparative HPLC, it was decided to continue without further improvement to the HWE reaction's selectivity.

#### 4.4 Synthesis of Rhabduscin

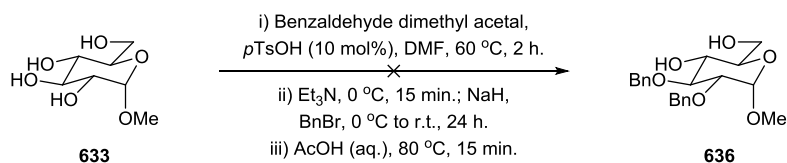
Due to the lack of commercial availability of Rhabduscin's required sugar residue **642**, a synthesis to this key intermediate was developed from the commercially available methyl- $\alpha$ -D-glucopyranose **633** involving: i) selective protection/deprotection of the hydroxyl groups C-4 and C-6 ii) selective elimination/substitution of C-6 and C-4 hydroxyl groups; (iii) reduction of azide/removal of *O*-benzyl protecting groups (Scheme 99). The synthesis of Rhabduscin **543** would then be completed using the route/methodology conceived for Byelyankacin **544**.





Scheme 99 Planned synthetic route to Rhabduscin **543**.

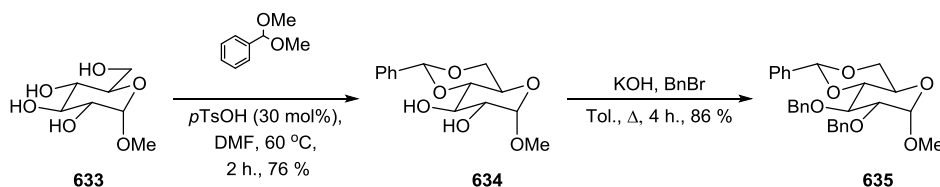
The first three steps in Rhabduscin's **543** synthesis were to prepare di-*O*-benzyl protected sugar species **636** via a series of selective protections and deprotections of the hydroxyl groups, with an investigation of the literature suggesting the feasibility of a one-pot approach. To this end, commercially available methyl- $\alpha$ -D-glucopyranose **633** was warmed to 30 °C and treated with benzaldehyde dimethyl acetal plus catalytic *p*-TsOH to facilitate selective acetal formation across the correctly orientated C-4 and C-6 hydroxyls (Scheme 100). This would then allow for the *in situ* di-benzylation of the remaining C-2 and C-3 hydroxyls via treatment with sodium hydride and benzylbromide, whilst subsequent exposure to aqueous acetic acid (post removal of organic solvent) was intended to effect the liberation of acetal protected C-4 and C-6 hydroxyls and afford di-*O*-benzyl protected sugar **636**. In reality however, this approach failed to yield the desired product **636**, with TLC analysis indicating full consumption of the starting materials and the formation of a complex mixture of products that could not be separated by chromatography.



Scheme 100 Failed one-pot synthesis of di-*O*-benzyl protected sugar **636**.

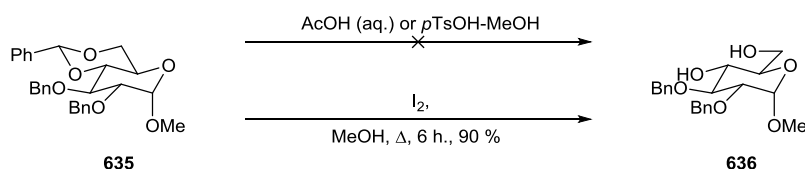
In light of this failure, it was decided to approach the synthesis of **636** step-wise (Scheme 101) since this would enable proper isolation of the intermediate products, hopefully improving the efficiency of the operations. Acetal protection of the C-4 and C-6 hydroxyls in methyl- $\alpha$ -D-glucopyranose **633** was again realised using benzaldehyde dimethyl acetal and 30 mol% of *p*-

TsOH (Scheme 101). Heating this mixture at 60 °C for 2 h. followed by neutralisation with triethylamine and aqueous workup afforded sugar acetal **634** that was easily purified on large scale by trituration with petroleum ether washings effectively removing excess benzaldehyde dimethyl acetal. The remaining two hydroxyls were then benzyl protected upon refluxing a suspension of potassium hydroxide, benzyl bromide and sugar acetal in toluene, affording relatively pure di-benzyl protected acetal **635** in excellent yield post aqueous work-up.



Scheme 101 Synthesis of di-benzyl protected acetal **635**.

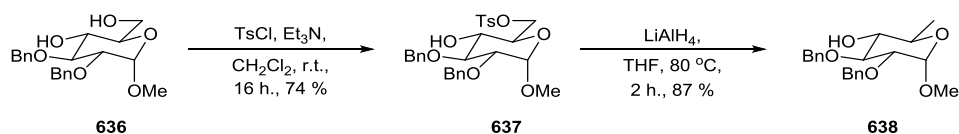
Attempts to remove the acetal protecting group of **635** using various acidic conditions failed to generate di-*O*-benzyl protected sugar **636** in any appreciable yield (Scheme 103). Increasing the stoichiometry/temperature of reaction had little effect. Refluxing of **635** with catalytic iodine and methanol according to the procedure first described by Sletten and Liotta (Scheme 102) did however prove successful, affording the required di-*O*-benzyl protected sugar **636** in 90% yield.<sup>199</sup>



Scheme 102 Synthesis of di-*O*-benzyl protected sugar **636**.

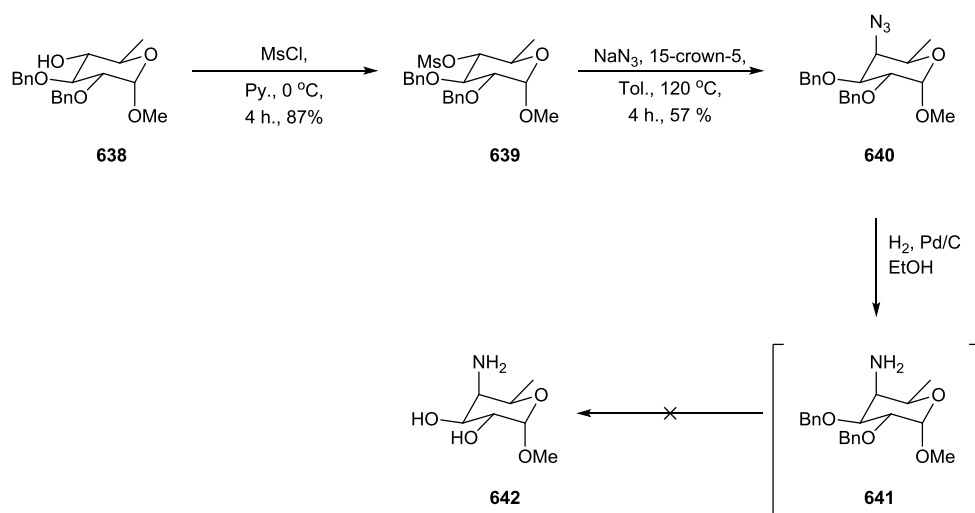
With the synthesis of the di-benzyl protected fragment **636** completed, work could then commence on the elimination/reduction operations required for the synthesis of sugar residue **642** (Scheme 103), which in turn would then enable the synthesis of Rhabduscin **543** *via* the methodology developed for the synthesis of Byelyankacin **544**. Di-benzyl protected sugar **636** was regioselectively tosylated at its primary C-6 hydroxyl group using tosylchloride and triethylamine base, with the afforded *O*-tosylate **637** undergoing hydride reduction upon treatment with lithium aluminium hydride. This effectively yielded the required methyl functionality of **638** with modification of the hydride reduction's work up such that the

reaction was quenched with aqueous ammonium chloride instead of water resulting in a 20% increase in reaction yield.



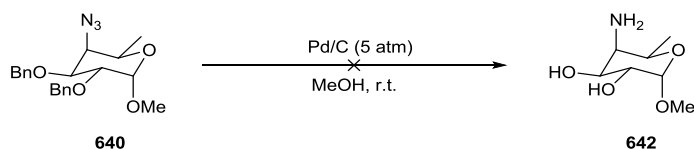
Scheme 103 Generation of the methyl group required for Rhabduscin.

To complete the synthesis of our desired *pseudo* starting material **642** the generation of the C-4 amine functionality and eventual removal of the remaining benzyl protecting groups had to be accomplished. To this end, secondary alcohol **638** was rapidly mesylated upon dissolving in ice cold pyridine and treating with methanesulfonylchloride to afford *O*-mesylate **639** (Scheme 104). Reaction of this leaving group containing species **639** with sodium azide, resulted in an  $\text{S}_{\text{N}}2$  inversion about the C-4 centre thereby affording axial azido sugar species **640**. The addition of crown ether was believed to increase the rate of this reaction by abstracting the sodium cation, therefore enhancing the nucleophilicity of the resulting aza-anion. It had been hoped that the amino-alcohol species **642** would simply be realised upon hydrogenation, with the presence of a palladium catalyst in a hydrogen atmosphere reducing the azide to the corresponding amine **641**, whilst concomitantly de-benzylating the protected C-2 and C-3 hydroxyls. However complete reduction and deprotection of hydroxyls failed to occur under these conditions and it was suggested that whilst the initial azide reduction may have occurred rapidly, analysis of the  $^1\text{H}$  NMR spectrum confirmed the presence of benzyl species **641** (Scheme 104).



Scheme 104 Synthesis of azide **640** and subsequent difficulty with hydrogenation.

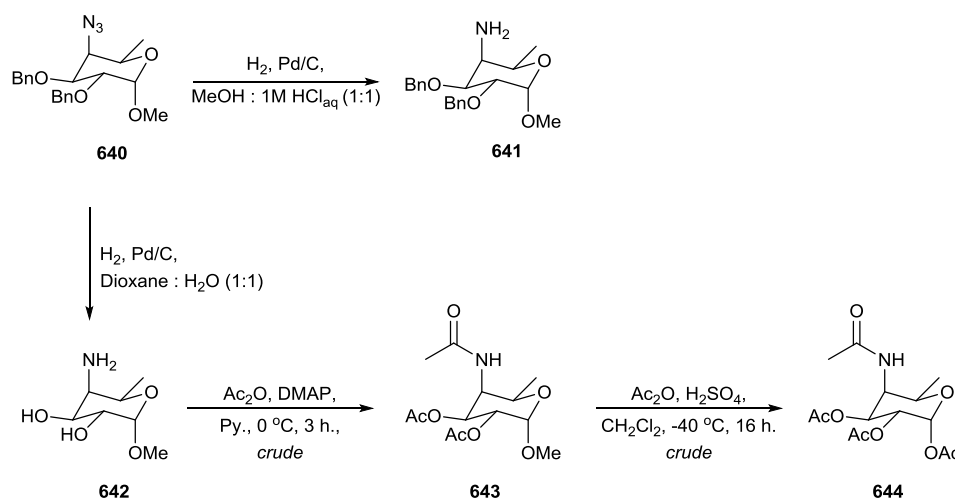
To establish more forcing reaction conditions, the azide **640** was placed in a bomb reactor and the hydrogen pressure increased from 1 to 5 atmospheres. However, the pressurised hydrogen atmosphere had a detrimental effect, failing to produce the desired reaction products, instead producing an uncharacterisable mixture of random products (Scheme 105). Providing additional kinetic energy to the reactants by repeating the atmospheric hydrogenations at the higher temperature of  $40\text{ }^{\circ}\text{C}$  had no effect.



Scheme 105 Failed "bomb" hydrogenation.

The use of acid containing solvent mixtures did however prove worthwhile, and whilst the use of aqueous hydrochloric acid in methanol successfully reduced the azide **640** to amine **641**, analysis of the  $^1\text{H}$  NMR spectrum confirmed that the benzyl groups had unfortunately remained attached (Scheme 106). Pleasingly, a mixture of dioxane and water (kept at pH 6) was found optimal for reduction and the concomitant de-benzylation of **640** to *pseudo* amino-alcohol starting material **642**. TLC analysis confirmed the presence of a new, highly polar product that contained no aromatic or *O*-benzyl protons in its  $^1\text{H}$  NMR spectrum. Despite a very poor yield for this reaction, we were keen to achieve the synthesis of per-acetate derivative **644** since this would provide us with an intermediate that was directly comparable to the Byelyankacin synthesis. The small amount of crude di-hydroxy-amine **642** that we had

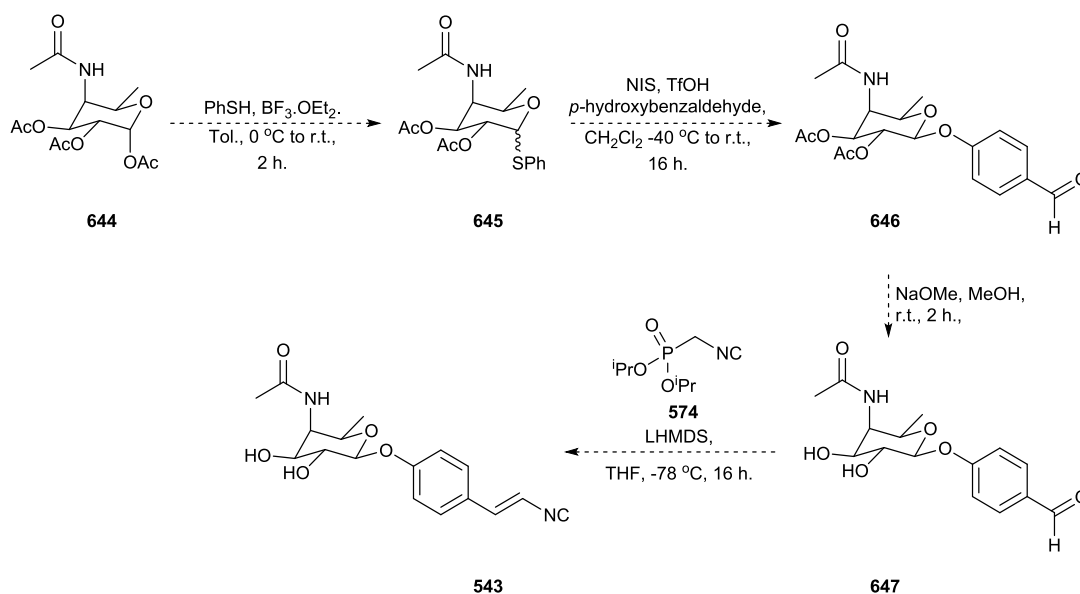
characterised was acetylated under the standard DMAP catalysed conditions to afford amide **643** (Scheme 106), the  $^1\text{H}$  NMR spectrum of this species clearly showing the addition of two *O*-acetate  $\text{CH}_3$  singlets at 2.11 and 2.08 ppm, plus an additional singlet at 2.00 ppm for the amide  $\text{CH}_3$  group. This amide **643** was then further exposed to acetic anhydride, this time in the presence of sulphuric acid, to facilitate replacement of the methyl ether functionality with a third and final *O*-acetate. Although our sugar sample was becoming rapidly depleted, we were able to obtain a crude  $^1\text{H}$  NMR of this per-acetate **644**, the spectrum clearly showing replacement of the methoxy singlet at 3.39 ppm with a new *O*-acetate singlet at 1.95 ppm.



Scheme 106 Successful hydrogenation protocol and subsequent per-acetylation.

Unfortunately, no success was had at effectively scaling our hydrogenation protocol, and thus significant quantities of di-hydroxy amine **642** were never obtained. Regrettably, the deadline for this Ph.D was imminent, and therefore the synthesis of Rhabduscin **543** is yet to be completed. To realise this goal, we obviously need to improve our hydrogenation protocol such that we may access significant quantities of di-hydroxy amine **642**, and subsequently convert this to per-acetate **644**. Since this per-acetate provides a synthetic comparison to the synthesis of Byelyankacin **544** it was our intention to subject per-acetylated sugar **644** to the methodology that had been previously developed for the synthesis of Byelyankacin **544**. Reaction of this per-acetylated fragment **644** with thiophenol and boron trifluoride diethyl etherate would again be effective at functionalising the sugar fragment with a leaving group (Scheme 107). This reaction would afford thioether **645** that upon treatment with NIS, triflic acid and *p*-hydroxybenzaldehyde **540** would selectively glycosylate **645** to yield aldehyde **646** as its  $\beta$ -anomer due to neighbouring group participation of the adjacent acetate. Selective hydrolysis of glycosyl benzaldehyde **646** with sodium methoxide would have resulted in

deprotection of the two *O*-acetates whilst leaving the more stable amide functionality untouched, yielding the final intermediate **647** in near quantitative yield. Finally, we intended to achieve Rhabduscin **543** upon reaction with LHMDS deprotonated *i*Pr-PhosMIC **574** (Scheme 107).



Scheme 107 Envisaged route to Rhabduscin **543**.

## 4.5 Conclusions

With the instability of phenol vinyl-isocyanide **542** preventing its use as a coupling partner for rhamnopyranoside synthesis, we instead developed a synthesis of glycosidic aldehydes that would subsequently undergo HWE homologation to afford Byelyankacin **544** and Rhabduscin **543**. HWE reaction of the pyranoside-aldehyde **617** with our isopropyl phosphonate reagent effected Byelyankacin's **544** synthesis with a 93 : 7 preference for the desired *trans*- isomer. For Rhamnose, synthesis of the starting amine-derived sugar **642** was required, it being our intention to subject this species to the synthetic operations previously used for Byelyankacin's **544** synthesis. Whilst able to achieve the synthesis of this 'starting sugar' **642**, the hydrogenation protocol used in the final step of its synthesis was un-reliable and low yielding, hampering our efforts towards Rhabduscin **543**. It is hoped that we will have time in the near future to overcome this problem and then proceed on gram scale towards Rhabduscin **543**, confident that the synthetic procedures used for Byelyankacin **544** will work equally as well for Rhabduscin's synthesis.

## 5 Synthesis of Paerucumarin

### 5.1 Introduction

When researching the biosynthesis of vinyl-isocyanides Byelyankacin **544**, Rhabduscin **543** and their agylcone (phenol vinyl-isocyanide **542**), an additional ‘pseudo’ vinyl-isocyanide derived natural product Paerucumarin **19** (Figure 46) was brought to our attention. 3-indole vinyl-isocyanide and phenol vinyl-isocyanide **542** (and therefore Byelyankacin **544** and Rhabduscin **543**) are biosynthesised by the action of isonitrile synthases (*isnA/B*) upon amino acids tryptophan **14** and tyrosine **11** (Figure 8). For this species, alternative *Pvc* gene clusters are expressed/replaced *isnA* and B, transforming tyrosine **11** into a 3-isocyano-6,7-dihydroxycoumarin **19** natural product.<sup>20,200</sup> For its biosynthesis, *PvcA* first acts as an isonitrile synthase, transforming the amine functionality of tyrosine **11** into its related isocyanide **648**, which is then followed by *PvcB* promoted elimination to afford vinyl-isocyanide **559**. Paerucumarin **19** is generated by *PvcC/D* oxidation of phenol **559** to diquinone **649** and its subsequent cyclisation/aromatisation.

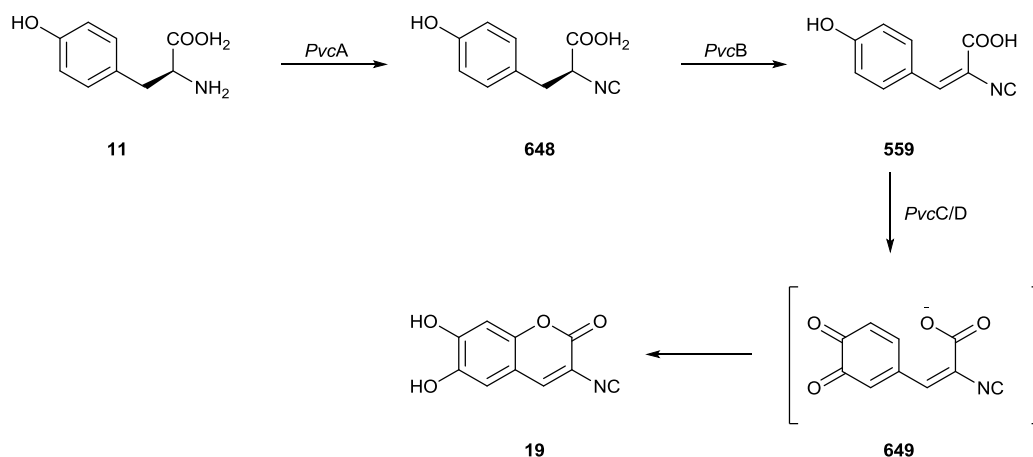


Figure 46 Biosynthesis of Paerucumarin **19**.

Based on the findings of the literature review, and indeed our previous syntheses of aliphatic isocyanides, it seemed highly likely that our synthesis would involve dehydration of Paerucumarin's **19** parent formamide. We anticipated that since the formamide's double bond would be part of the lactone ring, it would render this species stable enough to the harsh conditions required to effect its dehydration. Interestingly, this 3-formido-6,7-

dihydroxycoumarin is known as Pseudoverdin **700**, a secondary metabolite of pPYP17 mutated *Psuedomonas aeruginosa* bacteria usually responsible for the biosynthesis of the Pyoverdins **701** (Figure 47).<sup>201</sup> This mutation produces Pseudoverdin **700** from dihydroxylation of tyrosine, with the installation of the C-2 phenol enabling facile ring closure through action upon the amino acid's carboxyl group.<sup>202</sup>

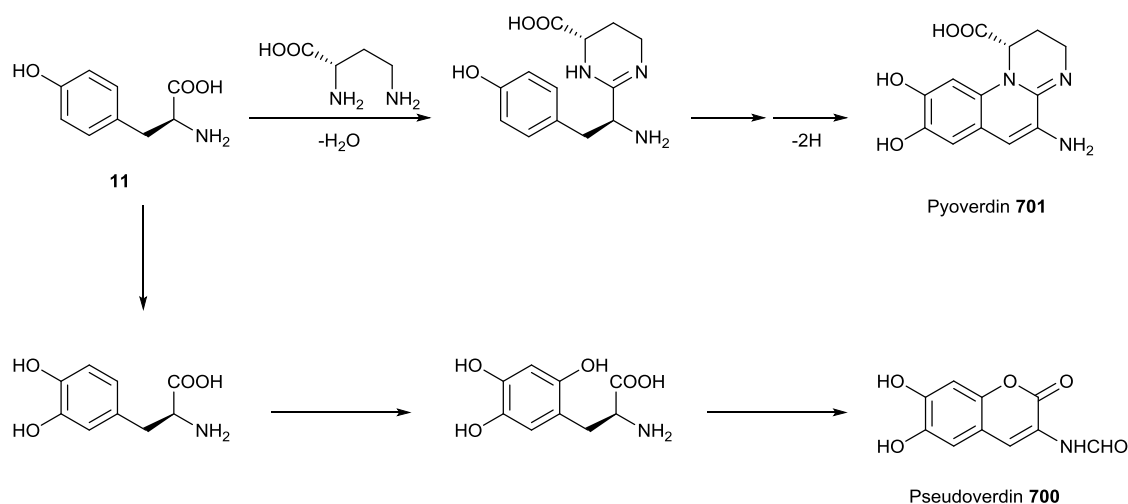


Figure 47 Biosynthesis of Pseudoverdin **700** and related Pyoverdin **701**.<sup>201,202</sup>

Since a synthesis of these natural products is yet to be reported, we were excited to develop an efficient synthesis of Paerucumarin **19** *via* Pseudoverdin **700**. Owing to the structural difference of Paerucumarin **19** with respect to phenol vinyl-isocyanide **542** we anticipated these coumarin species to be biologically inert.

## 5.2 Synthesis of Pseudoverdin and Paerucumarin

When researching the synthesis of coumarin systems it became evident that the most popular strategy involved condensation of the corresponding salicylaldehyde with an appropriately functionalised methylene ester. Of particular interest was the method reported by Shen and co-workers, for their catalytic condensation of salicylaldehydes with ethyl isocyanoacetate **702** provides a direct route to 3-formaido-coumarins (Figure 48).<sup>203</sup> For his mechanism, Shen implies that copper's soft nature enables it to bind the isocyanide of the ester reagent, lowering the pKa of the methylene protons such that the pyridine may



deprotonate here. The resultant enolate then attacks the salicylaldehyde's carbonyl functionality, with the generated hydroxyl anion then undergoing hydrogen exchange with the phenolic proton, an equilibrium process that serves to generate a nucleophilic oxygen for lactone formation. Post cyclisation, the remaining  $sp^2$  hybridised alcohol group clips onto the isocyanide carbon, affording an oxazole-like intermediate that rearranges to yield both the alkene and formamide functionality of the coumarins (Figure 48).<sup>203</sup>

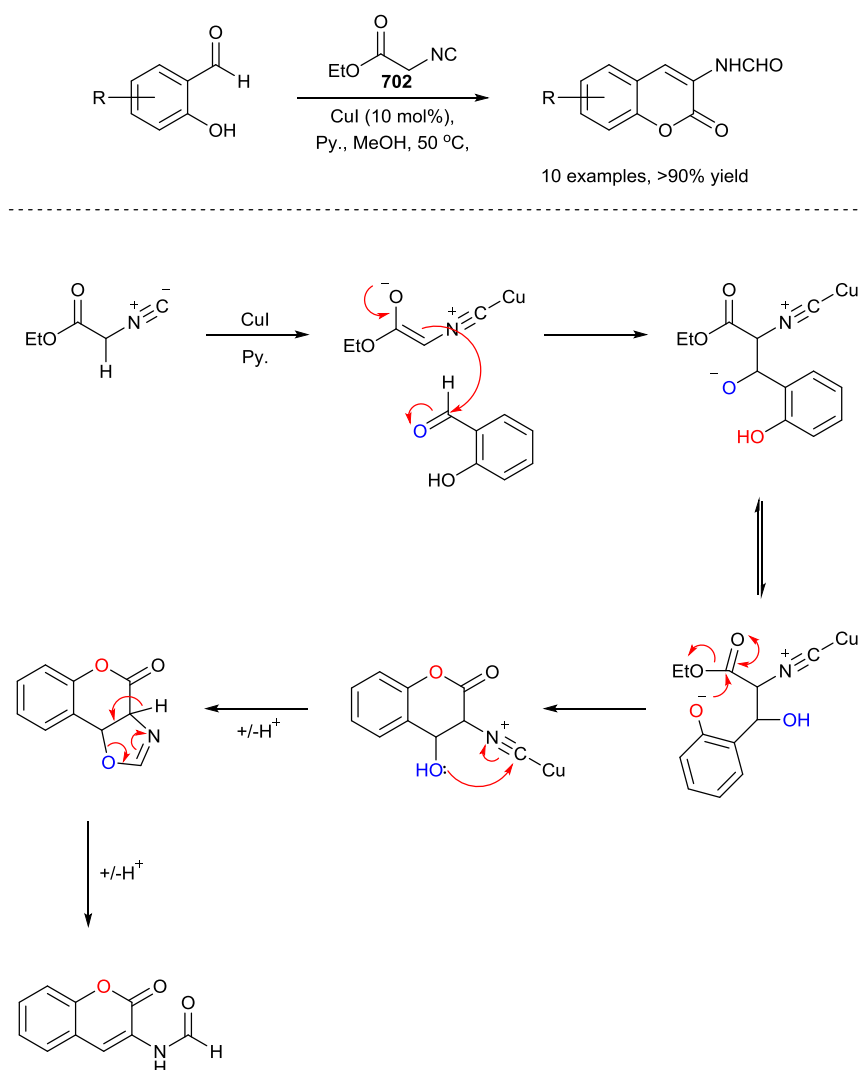
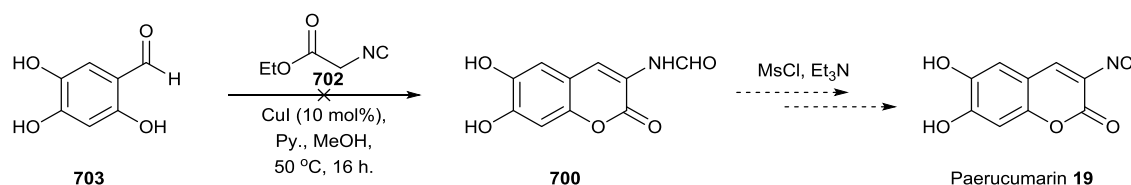


Figure 48 Meng's protocol, and proposed mechanism, for the synthesis of 3-formido coumarins.<sup>203</sup>

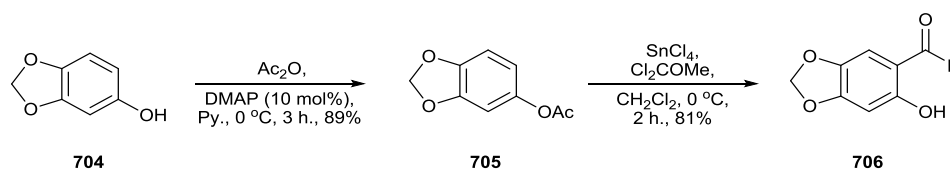
With this in mind, it was our intention to condense 2,3,5-trihydroxybenzaldehyde **703** with ethyl isocyanoacetate **702**, as per Shen's protocol, and then dehydrate Psuedoverdin **700** with methane sulfonylchloride to afford the isocyanide functionality of Paerucumarin **19**. At this time we were unsure as to whether the diol functionality would need to be *O*-silyl protected for the dehydration step, and this was something we intended to explore with a series of test reactions post-cyclisation. To start with, 2,3,5-trihydroxybenzaldehyde **702** was

suspended in methanol, treated with copper iodide, pyridine and ethyl isocyanoacetate **702** and left to stir at 50 °C overnight (Scheme 108).



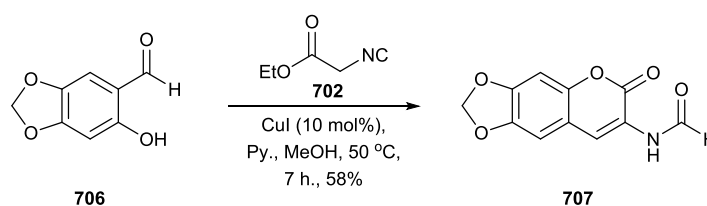
Scheme 108 – Attempted application of Meng's protocol to 2,3,5-trihydroxybenzaldehyde **703**.

Unfortunately no success was to be had using the highly expensive *tris*-hydroxybenzaldehyde **703**, with multiple attempts at this reaction returning the starting aldehyde. We speculated that it was the presence of 'surplus' hydroxyls and their competitive deprotonation that was to blame for the failed cyclisation of this salicylaldehyde. Furthermore, this may explain why there were no hydroxyl-coumarin examples published in Shen's paper.<sup>203</sup> To overcome this complication, we sought to find an alternative salicylaldehyde with the C-4 and C-5 hydroxyls protected, leaving the C-2 hydroxyl free to participate in the condensation reaction without competition. Subsequent research brought benzodioxolane **706**, and its synthesis from readily available sesamol **704**, to our attention.<sup>204</sup> Whilst the strategies for removing the methylene ether protecting group are limited to the use of highly acidic boron tribromide, we assumed that an aromatic formamide would be able to survive these unavoidably harsh conditions. Using the chemistry reported by Nichols and co-workers,<sup>204</sup> the free-phenol functionality of sesamol **704** was first protected as its *O*-acetate *via* treatment with catalytic DMAP and acetic anhydride, using pyridine as a solvent and base. The resultant *O*-acetate **705** then underwent a Friedel-Crafts formylation, with concomitant acetate hydrolysis, through the action of tin tetrachloride and dichloromethyl methyl ether to afford the desired benzodioxolane functionalised salicylaldehyde **706** (Scheme 109).



Scheme 109 Synthesis of suitable 'protected' salicylaldehyde derivative **706**.<sup>204</sup>

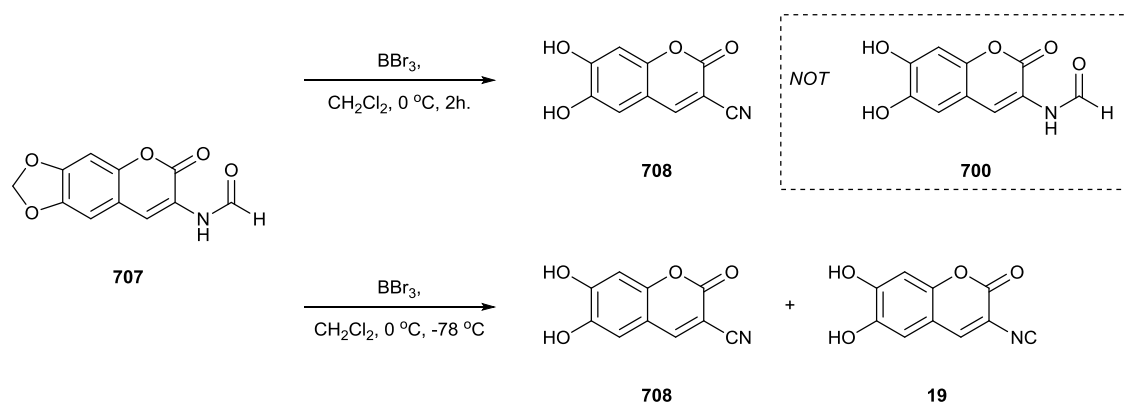
This benzodioxolane containing salicylaldehyde **706** was then subjected to Shen's coumarin synthesis *via* condensation with ethyl isocyanoacetate **702** in the presence of catalytic copper iodide and pyridine base (Scheme 110).<sup>203</sup> After stirring this combination for 7 h. in 50 °C methanol, the resultant pink precipitate was collected by filtration and characterised as the desired (and novel) 3-formido-coumarin **707**. The four singlet proton peaks observed in <sup>1</sup>H NMR spectrum of **707** were found to be comparable in shift to those recorded by Shen for the structurally similar 3-formido-6,7-dimethoxycoumarin.<sup>203</sup> Definitive confirmation of its structure came from analysis of its mass spectrum and interpretation of infra-red stretches for the amide and lactone functionalities of **707**.



Scheme 110 Coumarin synthesis *via* condensation reaction of 'protected' salicylaldehyde **706**.

The sensitivity/intolerance of isocyanides to harsh and/or acidic conditions made it necessary to carry out boron tribromide facilitated ether cleavage prior to dehydration of the formamide functionality, affording Pseudoverdin **700** in the process. The addition of excess boron tribromide to ether **707** successfully cleaved the methylene *bis*-ether functionality, as noted by loss of the  $\text{CH}_2$  ether peak at 6.14 ppm in the <sup>1</sup>H NMR spectrum (Scheme 111). Interestingly, we also noted the loss of the peaks at 10.12 and 8.59 ppm (formamide NH and CHO), suggesting that we hadn't synthesised Pseudoverdin **700** as expected. Initially, it was assumed that the electron-deficient nature of the boron had served to concomitantly dehydrate the formamide by a similar mechanism to that of methanesulfonylchloride facilitated dehydration. However, we had not accidentally synthesised Paerucumarin **19** because the infra-red spectrum for the isolated product did not contain the characteristic isocyanide stretch around  $2100\text{ cm}^{-1}$ , instead we observed a nitrile stretch at  $2251.71\text{ cm}^{-1}$ . Further evidence for the presence of a nitrile was found in the <sup>13</sup>C NMR, with the 11<sup>th</sup> carbon not appearing as a broad quadrapolar shift around 160 ppm, but as a nitrile resonance at 110 ppm. The rearrangement of aromatic isocyanides to their corresponding nitrile has been reported at elevated temperatures.<sup>205</sup> Therefore we believe that the harsh boron tribromide conditions have served to concomitantly cleave the ether group of **707** and dehydrate its formamide **700** functionality to initially yield Paerucumarin

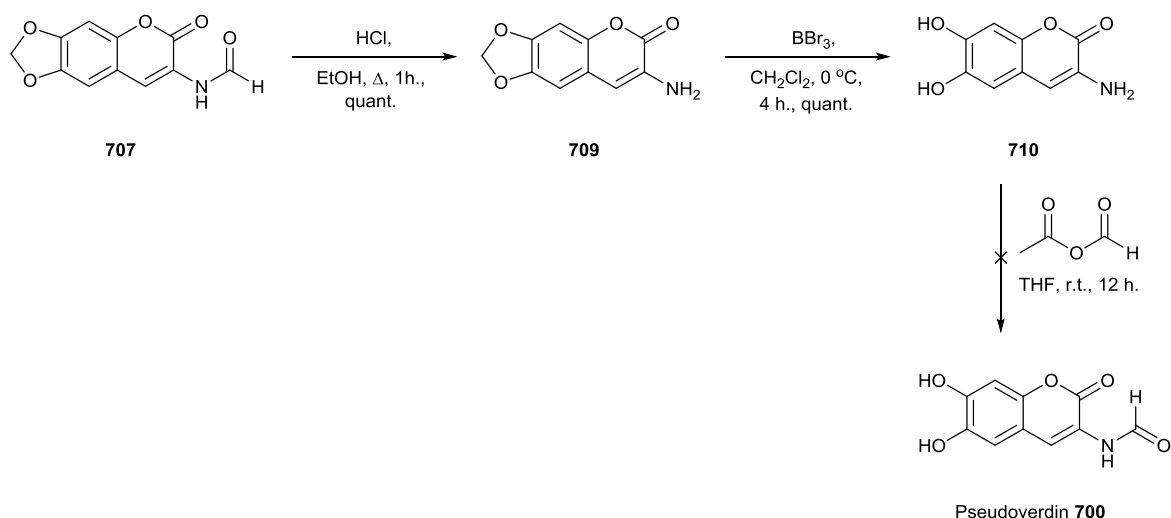
**19.** However, owing to the harsh reaction conditions, it appears that the isocyanide functionality has subsequently rearranged to the corresponding nitrile species **708**. By reducing the reaction temperature to  $-78\text{ }^{\circ}\text{C}$ , we were able to isolate an inseparable mixture of nitrile-coumarin **708** and Paerucumarin **19**, the IR of this reaction mixture clearly contains both the nitrile peak ( $2981.19\text{ cm}^{-1}$ ) and isocyanide peak ( $2124.79\text{ cm}^{-1}$ ).



Scheme 111 Problematic ether cleavages of formido-coumarin **707**.

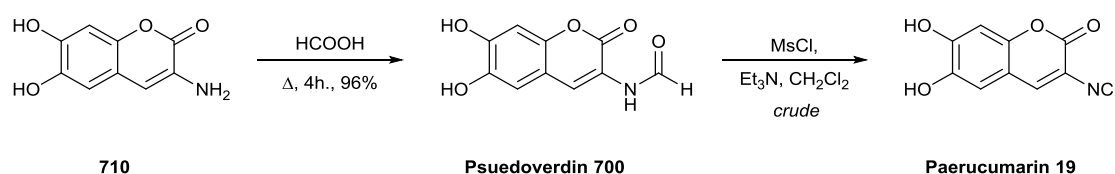
To circumnavigate this problem, it was decided to mask formamide **707** as its parent amine, and then attempt to cleave the ether group using boron tribromide, reformylating the amine if successful. To this end, formamide **707** underwent acidic hydrolysis using a 10 : 1 mixture of ethanol and aq. HCl, with the resultant amine **709** then being treated with boron tribromide. As a precaution, the amine was cooled to  $-78\text{ }^{\circ}\text{C}$  for the deprotection and by monitoring the reaction we found the ether group to be effectively cleaved after 3 h., affording clean dihydroxy-amino-coumarin **710** in near quantitative yield over the two steps. The poor solubility of this dihydroxy-amino-coumarin **710** in solvents other than water or alcohol proved problematic for its formylation, with our usual approach using acetic formic anhydride in THF proving ineffective.

## Synthesis of Paerucumarin



Scheme 112 Progress towards Paerucumarin **19** via Pseudoverdin **700**.

Pleasingly however, refluxing amine **710** in formic acid achieved its formylation, thus providing the first synthesis of Pseudoverdin **700**. Finally, the formamide functionality of **700** was dehydrated using methanesulfonylchloride and pyridine, affording Paerucumarin **19** and an inseparable unknown impurity (Scheme 113). The spectra of Pseudoverdin **700** and crude  $^1\text{H}$  NMR spectrum of Paerucumarin **19** could be validated against those reported by the isolation teams.<sup>20,201</sup>



Scheme 113 Synthesis of Paerucumarin **19** via Pseudoverdin **700**.

## 5.3 Conclusions

Pseudoverdin **700** was synthesised from a dihydroxy-3-amino-coumarin species **707** that was accessed using an elegant literature condensation of salicylaldehyde species **706** and ethyl isocyanoacetate **702**. Furthermore, we demonstrated that Pseudoverdin may be dehydrated to Paerucumarin and work is on-going to cleanly isolate this isocyanide derived natural product *via* fractional crystallisation.

## 6 Experimental

### 6.1 General Considerations and Procedures

Commercially available solvents and reagents were obtained from Sigma-Aldrich Company Ltd., Fisher Scientific Ltd., Alfa Aesar or Fluorochem Ltd. and were used without further purification. 'Petroleum ether' refers to the fraction of petroleum ether boiling in the range of 40-60 °C. All reactions were performed in oven-dried apparatus, whilst anhydrous solvents were obtained from an Innovative Technology Inc. PS-400-7 solvent purification system. Phosphate buffer refers to the pH 7 buffer made by mixing dibasic sodium phosphate (61.5 mL, 0.5M) and monosodium phosphate (38.5 mL, 0.5M).

Analytical thin layer chromatography was performed using commercially available aluminium backed plates coated with Merck G/UV254 neutral silica. Plates were visualised under UV light (at 254 nm) or by staining with either iodine adsorbed onto silica or phosphomolybdic acid followed by heating. Flash chromatography was performed using chromatography grade silica, 60 Å particle size 35-70 microns from Fisher Scientific.

<sup>1</sup>H NMR spectra were recorded at 500 MHz, 400 MHz 300 MHz or 250 MHz and <sup>13</sup>C{<sup>1</sup>H} spectra were recorded at 125 MHz 100 MHz or 75 MHz on a Brüker Avance 500, 400 or 300 spectrometer respectively. Chemical shifts,  $\delta$ , are quoted in parts per million and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; dq doublet of quartets; m, multiplet; pent, pentet; td, triplet of doublets; ddd, doublet of doublet of doublets; app, apparent and br, broad. Coupling constants, *J*, are quoted to the nearest 0.1 Hz.

High resolution mass spectra were recorded on a Brüker Daltonics microTOF spectrometer with an electrospray source and external calibration. Masses were recorded in positive electrospray ionisation mode and were introduced by flow injection. Masses are accurate to 5 ppm and data was processed using Data Analysis software from Brüker Daltonics. Compounds

containing an isocyanide or trichloroacetimidate were unable to withstand ionisation, and therefore mass spectra were not obtained for these species.

Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer, using a Universal ATR accessory for sampling, with only selected absorbances quoted as  $\nu$  in  $\text{cm}^{-1}$ . Optical rotations were recorded on an Optical Activity Ltd AA-10 automatic polarimeter with a path length of 1 dm; concentrations (c) are quoted in g/100 mL.

Purification/analysis of compounds by HPLC was performed on an Agilent technologies 1260 infinity semi-preparative HPLC using an eclipse XDB-C18 5  $\mu\text{m}$ , using UV light (at 254 nm) for detection.

#### General Procedure 1: Arbuzov Reactions

The appropriate tri-alkyl phosphite (1.3 equiv.) is added to a suspension of *N*-((dimethylamino)methyl)formamide **565** (1.0 equiv.) in EtOH (10  $\text{mLg}^{-1}$ ) under a nitrogen atmosphere, and the whole refluxed (bath temp. 100 °C) for 8 h. The EtOH is subsequently removed *in vacuo* and the EtOAc soluble components of the resulting residue are collected after filtration as the crude product(s).

#### General Procedure 2: Dehydration of formamides

A dry flask purged with nitrogen is charged with the desired formamide (1.0 equiv.), anhydrous  $\text{CH}_2\text{Cl}_2$  (10  $\text{mLg}^{-1}$ ) and  $\text{Et}_3\text{N}$  (9 equiv.). The whole is cooled to -78 °C and  $\text{MsCl}$  (3.0 equiv.) added dropwise. After stirring for 16 h., the reaction is quenched by the addition of aq.  $\text{NaHCO}_3$ , and extracted twice with  $\text{CH}_2\text{Cl}_2$ , the organics then being dried over  $\text{MgSO}_4$  and concentrated (post filtration) to afford the crude reaction product(s).

### General Procedure 3: *O*-silylation protocol

The appropriate hydroxyl species (1 equiv.) and imidazole (1.1 equiv.) are dissolved in  $\text{CH}_2\text{Cl}_2$  ( $10 \text{ mLg}^{-1}$ ) at r.t. and *tert*-butyl-di-methylsilylchloride (1.1 equiv.) added portion wise over 10 min. After stirring for 16 h., the reaction is quenched with  $\text{H}_2\text{O}$  and extracted thrice with  $\text{CH}_2\text{Cl}_2$ . The combined organics are washed with brine, dried over  $\text{MgSO}_4$  and concentrated (post filtration) to afford the crude reaction product(s).

### General Procedure 4: *N*-formylation reactions

The required amine (1.0 equiv.) is dissolved in THF ( $5 \text{ mLg}^{-1}$ ) and cooled to  $0^\circ\text{C}$  in an ice bath. Acetic formic anhydride (1.1 equiv.) is then added dropwise and the whole is stirred for 1 to 2 h., before THF and excess acetic formic anhydride are removed by rotary evaporation to afford the crude product(s).

### General Procedure 5: *O* and *N*-Acetylation of sugars

Over a period of 30 min., ice cold acetic anhydride ( $10 \text{ mLg}^{-1}$ ) is added dropwise to a mixture of the appropriate alcohol/amine (1.0 equiv.) and DMAP (10 mol%) dissolved in pyridine ( $20 \text{ mLg}^{-1}$ ), the whole then being stirred for 3 h. at  $0^\circ\text{C}$  under nitrogen. The reaction mixture is then concentrated (post filtration) *in vacuo* and the evaporation residue dissolved in toluene ( $50 \text{ mLg}^{-1}$ ) and washed twice with aq. 1M HCl, once with water, once with brine and then dried over  $\text{MgSO}_4$  and concentrated to afford the crude product(s).

### General Procedure 6: Synthesis of thioethers

Boron trifluoro ethyl etherate (1equiv.) is added to a  $0^\circ\text{C}$  solution of the appropriate sugar acetate (1 equiv.) and thiophenol (1 equiv.) dissolved in anhydrous toluene ( $5 \text{ mLg}^{-1}$ ). The reaction mixture is then warmed to r.t. and stirred for 2 h. before being cooled back to  $0^\circ\text{C}$



and quenched with aq. 1M NaOH (5 mLg<sup>-1</sup>). Following extraction of the aqueous phase with two portions of toluene, the combined organics were washed with brine, dried over MgSO<sub>4</sub> and concentrated (post filtration) to afford the crude product(s).

#### General Procedure 7: Glycosidation reactions

Triflic acid (0.25 equiv.) and *N*-iodosuccinamide (1.0 equiv.) are added in quick succession to a -40 °C solution of the appropriate sugar derived thioether (1.0 equiv.) and 4-hydroxybenzaldehyde (1 equiv.) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mLg<sup>-1</sup>) under a nitrogen atmosphere. After stirring at -40 °C for 16 h., Et<sub>3</sub>N and CH<sub>2</sub>Cl<sub>2</sub> are added and the whole mixture filtered through a pad of celite. The organic filtrate is then washed with aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and brine, with the crude product(s) being afforded upon drying the organic phase over MgSO<sub>4</sub> and concentrating (post filtration) *in vacuo*.

#### General Procedure 8: Hydrolysis of acetates

NaOMe (0.5 M in MeOH) (0.25 equiv.) is added dropwise to the appropriate acetate (1 equiv.) dissolved in MeOH (5 mLg<sup>-1</sup>) and stirred for 3 h. under nitrogen before being neutralised by pouring through a plug of Amberlite IR-120 resin and the resultant mother liquor evaporated to afford the crude product(s).

#### Schöllkopf HWE Protocol

Diethyl isocyanomethylphosphonate (1 equiv.) is stirred with NHMDS (1.1 equiv.) in THF (10 mLg<sup>-1</sup>) and the mixture held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate aldehyde (1 equiv.) is added and the whole allowed to warm up to r.t. over 16 h. The reaction is quenched using phosphate buffer, partitioned with EtOAc and the organic portion dried over MgSO<sub>4</sub> and concentrated (post filtration) to afford the crude product(s).

**Optimised HWE Procedure A: Synthesis of phenol derived isocyanides**

Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDs (1.2 equiv.) in THF (10 mL<sup>-1</sup>) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate *O*-silyl protected hydroxybenzaldehyde (1 equiv.) is added and the whole immediately warmed up to room temperature before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc, dried over MgSO<sub>4</sub> and concentrated (post filtration). The resultant oil is then dissolved in ethanol (10 mL approx), KOH added (2 pellets) and stirred at room temperature for 2 h. before being concentrated, and the resultant oil partitioned between aq. NaHCO<sub>3</sub> and EtOAc. The organics are dried over MgSO<sub>4</sub> and concentrated (post filtration) to afford the crude phenol vinyl-isocyanide(s).

**Optimised HWE Procedure B: Synthesis of hetroaromatic derived isocyanides**

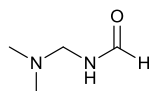
Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDs (2.2 equiv.) in THF (10 mL<sup>-1</sup>) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate hetroaromatic carbaldehyde (1 equiv.) is added and the whole immediately warmed to r.t. before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc before MgSO<sub>4</sub> is added and the whole concentrated (post filtration) to afford the crude hetroaromatic vinyl-isocyanide(s).

**Optimised HWE Procedure C: Synthesis of non phenolate, non-heteroaromatic derived isocyanides**

Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDs (1.2 equiv.) in THF (10 mL<sup>-1</sup>) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate benzaldehyde (1 equiv.) is added and the whole immediately warmed up to r.t. before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc before MgSO<sub>4</sub> is added and the whole concentrated (post filtration) to afford the crude product(s)

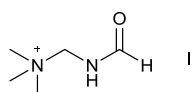
## 6.2 Compounds associated with the development of an *E*-selective Horner Wadsworth Emmons Reaction (chapter 2).

### *N*-((dimethylamino)methyl)formamide **564**<sup>206</sup>



Formaldehyde (37 wt.% in H<sub>2</sub>O, 128 mL, 1.25 mol) was added to a 0 °C mixture of formamide (50 mL, 1.25 mol) and dimethylamine (40 wt.% in H<sub>2</sub>O, 128 mL, 1.25 mol) and the resulting solution allowed to warm up to r.t. whilst stirring for 16 h. The reaction mixture was then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 75 mL), with the combined organics being dried over Na<sub>2</sub>SO<sub>4</sub> and the evaporation residue distilled (post filtration) to afford the desired compound as a colourless oil (65.2 g, 50% yield): b.p. 82 °C (2mmHg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 8.20 (1H, m, CHO), 7.11 (1H, br s, NH), 3.76 (2H, d, *J* = 5.1 Hz, NCH<sub>2</sub>N), 2.07 (6H, m, N(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 163.7, 63.4, 42.0; IR (film, cm<sup>-1</sup>): ν = 3314.23 (N-H), 1672.90 (C=O); HRMS (ESI) calcd for C<sub>4</sub>H<sub>11</sub>N<sub>2</sub>O [M+H]<sup>+</sup>: found *m/z* 103.0870, requires *m/z* 103.0871.

### 1-formamido-*N,N,N*-trimethylmethanaminium iodide **565**<sup>206</sup>

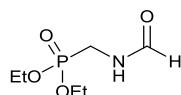


*N*-((dimethylamino)methyl)formamide **564** (15.0 g, 0.147 mol) and Et<sub>2</sub>O (200 mL) were cooled to 0 °C under Nitrogen, and iodomethane (22 mL, 0.442 mol) added. The whole was stirred for 2 h. before the Et<sub>2</sub>O was removed *in vacuo* to produce a white solid that was recrystallised from hot ethanol to afford the title compound as a white powder (21.2 g, 59% yield): m.p. 149-150 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 9.41 (1H, br s, NH), 8.57 (0.2H, m, CHO (*E* rotamer)), 8.45 (0.8H, s, CHO (*Z* rotamer)), 5.00 (0.4H, d, *J* = 7.0 Hz, NCH<sub>2</sub>N (*E* rotamer)), 4.75 (1.6H, d, *J* =

## Experimental

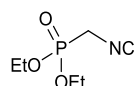
7.0 Hz, NCH<sub>2</sub>N (*Z* rotamer)), 3.12 (9H, s, N(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>c</sub> = 163.8, 54.1, 38.8 (m); IR (powder, cm<sup>-1</sup>): ν = 1696.83 (C=O); HRMS (ESI) calculated for C<sub>5</sub>H<sub>13</sub>N<sub>2</sub>O [M]<sup>+</sup>: found *m/z* 117.1041, requires *m/z* 117.1027

### Diethyl(formamidomethyl)phosphonate **566**<sup>207</sup>



Triethylphosphite (17.9 mL, 0.103 mol) was added to *N*-((dimethylamino)methyl)formamide **565** (19.26 g, 0.079 mol) in EtOH (200 mL) as required by general procedure 1. The crude reaction products were purified by silica gel chromatography [CH<sub>2</sub>Cl<sub>2</sub> : MeOH (95 : 5), R<sub>f</sub> 0.15] to afford title compound as a colourless oil (14.0 g, 87% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 7.80 (1H, s, CHO), 7.42 (1H, br s, NH), 3.92 (4H, m, OCH<sub>2</sub>CH<sub>3</sub>), 3.50 (2H, dd, *J* = 12.4, 6.2 Hz, PCH<sub>2</sub>), 1.11 (6H, t, *J* = 7.6 Hz, OCH<sub>2</sub>CH<sub>3</sub>, ); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>c</sub> = 162.2, 62.5 (m), 32.8 (d, *J* = 157.8 Hz), 16.3 (m); <sup>31</sup>P NMR (300 MHz, CDCl<sub>3</sub>) δ<sub>p</sub> = 22.8; IR (film, cm<sup>-1</sup>): ν = 3476.97 (N-H), 1694.00 (C=O); HRMS (ESI) calculated for C<sub>6</sub>H<sub>14</sub>NNaO<sub>4</sub>P [M+Na]<sup>+</sup>: found *m/z* 218.0575, requires *m/z* 218.0558.

### Diethyl isocyanomethylphosphonate **500**<sup>207</sup>

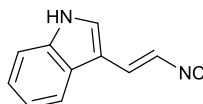


Diethyl (formamidomethyl)phosphonate **566** (3.6 g, 0.020 mol) was dehydrated according to general procedure 2 when dissolved in CH<sub>2</sub>Cl<sub>2</sub> (160 mL) and Et<sub>3</sub>N (26 mL, 0.185 mol) and treated with MsCl (4.8 mL, 0.061 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [petroleum ether : EtOAc (50 : 50), R<sub>f</sub> 0.28] to furnish title compound as a pale yellow oil (1.20 g, 32% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 4.24 (4H, m, OCH<sub>2</sub>CH<sub>3</sub>),

## Experimental

3.76 (2H, d,  $J = 15.8$  Hz,  $\text{PCH}_2$ ), 1.38 (6H, m,  $\text{OCH}_2\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}} = 160.8$ , 63.9, 37.6 (d,  $J = 156.7$  Hz), 16.3;  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{P}} = 14.1$ ; IR (film,  $\text{cm}^{-1}$ )  $\nu = 2152$  (IC)

### (*E*)-3-indole vinyl-isocyanide **17** (c.f. Antibiotic B731 **20**)<sup>155</sup>



#### Schöllkopf HWE Protocol.....

Diethyl isocyanomethylphosphonate **500** (244  $\mu\text{L}$ , 1.38 mmol) was stirred with NHMDS (1.0M in THF) (1.5 mL, 1.52 mmol) in THF (10 mL) according to Schöllkopf HWE Protocol and indole-3-carbaldehyde **499** (200 mg, 1.38 mmol) added. The resultant foul smelling residue was purified by flash column chromatography [petroleum ether : EtOAc (50 : 50),  $R_{\text{f}}$  0.28] to afford the title compound as a pale yellow oil (65 mg, 28% yield).

#### Optimised method.....

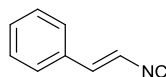
The title compound was prepared according to optimised HWE procedure B using indole-3-carbaldehyde **499** (200 mg, 1.38 mmol), diisopropyl isocyanomethylphosphonate **574** (340  $\mu\text{L}$ , 1.65 mmol) and LHMDS (1.0 M in THF) (3.30 mL, 3.31 mmol) in THF (10 mL). Purified by flash column chromatography [pentanes : EtOAc (50 : 50),  $R_{\text{f}}$  0.28] to furnish title compound as a pale yellow oil (125 mg, 57% yield). Selective for *E*-isomer.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}} = 8.42$  (1H, br s, NH), 7.68 (1H, d,  $J = 7.9$  Hz, ArH), 7.42 (1H, d,  $J = 7.9$  Hz, ArH), 7.34 (1H, d,  $J = 2.1$  Hz, ArH), 7.26 (2H, m, ArH), 7.13 (1H, d,  $J = 14.4$  Hz,  $\beta$  vinyl H),

## Experimental

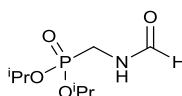
6.35 (1H, d,  $J = 14.4$  Hz,  $\alpha$  vinyl  $H$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}} = 163.0, 136.9, 130.2, 126.3, 124.6, 123.4, 121.4, 119.9, 111.8, 111.1, 107.1$ .

### (*E*)-(2-isocyanovinyl)benzene **568**<sup>155</sup>

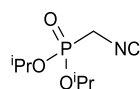


The title compound was prepared according to optimised HWE procedure C using benzaldehyde (50 mg, 0.47 mmol), diethyl isocyanomethylphosphonate **500** (100  $\mu\text{L}$ , 0.56 mmol) and LHMDS (1.0 M in THF) (0.57 mL, 0.56 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.90] to afford title compound as a black oil (33 mg, 35% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}} = 7.36$  (5H, m,  $ArH$ ), 6.95 (1H, d,  $J = 14.3$  Hz, (*E*)  $\beta$  vinyl  $H$ ), 6.28 (1H, d,  $J = 14.3$  Hz, (*E*)  $\alpha$  vinyl  $H$ ), 5.85;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}} = 165.3, 136.7, 129.9, 129.3, 126.7$ ; IR (film,  $\text{cm}^{-1}$ ):  $\nu = 2121$   $\text{cm}^{-1}$ .

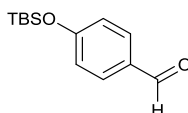
### Diisopropyl (formamidomethyl)phosphonate **573**



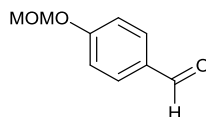
Triisopropylphosphite (16.7, 0.067 mol) was added to *N*-((dimethylamino)methyl)formamide **565** (11.0 g, 0.045 mol) in EtOH (110 mL) as required by general procedure 1. The crude reaction products were purified by silica gel chromatography [pentanes : EtOAc (50 : 50),  $R_f$  0.10] to afford the title compound as a colourless oil (8.50 g, 85% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}} = 8.20$  (1H, s,  $CHO$ ), 6.91 (1H, br s,  $NH$ ), 4.67 (2H, m,  $OCH$ ), 3.65 (2H, dd,  $J = 12.8, 0.8$  Hz,  $PCH_2$ ), 1.30 (12H, m,  $CH_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}} = 161.1, 71.7, 34.2$  (d,  $J = 158.8$  Hz), 24.0;  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{P}} = 21.1$ ; IR (film,  $\text{cm}^{-1}$ ):  $\nu = 3267.99$  (N-H), 1685.06 (C=O); HRMS (ESI) calculated for  $\text{C}_8\text{H}_{18}\text{NNaO}_4\text{P}$   $[M+\text{Na}]^+$ : found  $m/z$  246.0899, requires  $m/z$  246.0982.

**Diisopropyl (isocyanomethyl)phosphonate 574**

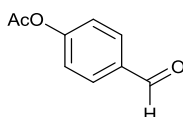
Diisopropyl (formamidomethyl)phosphonate (2.33 g, 0.015 mol) **573** was dehydrated according to general procedure 2 when dissolved in  $\text{CH}_2\text{Cl}_2$  (25 mL) and  $\text{Et}_3\text{N}$  (13 mL, 0.093 mol) and treated with  $\text{MsCl}$  (3.55 mL, 0.031 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes :  $\text{EtOAc}$  (50 : 50),  $R_f$  0.28] to furnish title compound as a pale yellow oil (1.57 g, 50% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 4.69 (2H, sept,  $J$  = 7.5 Hz, OCH) 3.64 (2H, d,  $J$  = 15.82 Hz,  $\text{PCH}_2$ ), 1.27 (12H, d,  $J$  = 7.5 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 160.0, 72.8, 38.2 (d,  $J$  = 156.9 Hz), 23.7;  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{P}}$  = 12.5; IR (film,  $\text{cm}^{-1}$ )  $\nu$  = 2151.22 (IC)

**4-((*tert*-butyldimethylsilyl)oxy)benzaldehyde 576<sup>208</sup>**

The title compound was prepared according to general procedure 3 using *p*-hydroxybenzaldehyde **560** (5.00 g, 0.041 mol), imidazole (8.37 g, 0.123 mol) and TBSCl (7.35 g, 0.049 mol) in  $\text{CH}_2\text{Cl}_2$  (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 9.86 (1H, s, CHO), 7.78 (2H, d,  $J$  = 8.5 Hz, ArH), 6.92 (2H, d,  $J$  = 8.5 Hz, ArH), 0.97 (9H, s,  $\text{SiC}(\text{CH}_3)_3$ ), 0.23 (6H, s,  $\text{SiC}(\text{CH}_3)_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 190.9, 161.5, 131.9, 130.5, 120.5, 25.6, 18.3, 4.4; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 1695.30 (C=O); HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{20}\text{O}_2\text{SiNa}$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  259.1145, requires  $m/z$  259.1131

**4-(methoxymethoxy)benzaldehyde 237**<sup>209</sup>

To *p*-hydroxybenzaldehyde (2.50 g, 0.021 mol) dissolved in anhydrous acetone (80 mL) was added  $K_2CO_3$  (11.60 g, 0.084 mol). Bromomethyl methyl ether (2.12 mL, 0.026 mol) was added dropwise and the whole was refluxed for 2 h. The reaction mixture was cooled to r.t. and filtered. The filtrate was concentrated *in vacuo* and purified by silica gel column chromatography [petroleum ether : EtOAc (70 : 30),  $R_f$  0.56] to yield title compound as a colourless oil (3.02 g, 88% yield).  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta_H$  = 9.88 (1H, s, CHO), 7.82 (2H, d,  $J$  = 8.7 Hz, ArH), 7.13 (2H, d,  $J$  = 8.7 Hz, ArH), 5.23 (2H, s,  $OCH_2O$ ), 3.47 (3H, s,  $OCH_3$ );  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ):  $\delta_C$  = 190.9, 161.2, 131.9, 130.7, 116.3, 94.1, 56.3; IR (film,  $cm^{-1}$ ):  $\nu$  = 1683.99 (C=O); HRMS (ESI) calcd for  $C_9H_{10}NaO_3$   $[M+Na]^+$ : found  $m/z$  189.0527, requires  $m/z$  189.0527

**4-formylphenyl acetate 578**<sup>210</sup>

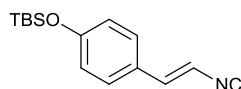
Acetyl chloride (2.89 g, 0.036 mol) was added dropwise to a mixture of *p*-hydroxybenzaldehyde **560** (3.00 g, 0.024 mol) and  $Et_3N$  (5.14 mL, 0.037 mol) dissolved in anhydrous THF (75 mL), the whole then being warmed to r.t. and stirred for 3 h. The reaction was worked up by quenching with aq.  $NH_4Cl$  and extraction with EtOAc (150 mL). The organics were washed with water (150 mL) and brine (150 mL) before being dried over  $MgSO_4$  and concentrated to give a pale oil. Purification by silica gel column chromatography [petroleum ether : EtOAc (70 : 30),  $R_f$  0.56] yielded title compound as a colourless oil (3.50 g, 89% yield).  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta_H$  = 9.92 (1H, s, CHO), 7.85 (2H, d,  $J$  = 8.63 Hz, ArH), 7.21 (2H, d,  $J$  =



## Experimental

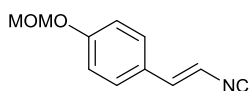
8.63 Hz, ArH), 2.26 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 190.9, 168.7, 155.4, 134.0, 131.2, 122.38, 21.2; IR (film, cm<sup>-1</sup>): ν = 1760.10 (C=O), 1696.76 (C=O); HRMS (ESI) calculated for C<sub>9</sub>H<sub>8</sub>O<sub>3</sub>Na [M+Na]<sup>+</sup>: found *m/z* 187.0364, requires *m/z* 187.0371,

### 4-((*tert*-butyldimethylsilyl)oxy)phenyl vinyl-isocyanide **579**



The title compound was prepared according to general procedure C from LHMDS (1.0 M in THF) (1.80 mL, 0.0024 mol), diethyl isocyanomethylphosphonate **500** (0.30 mL, 0.0013 mol) and *p*-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **576** (0.12 mL, 0.0012). The crude product was purified by kugelrohr distillation to give title compound as a brown oil (100 mg, 55%): b.p. 126 °C, 2mmHg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 7.28 (2H, d, *J* = 8.7 Hz, ArH), 6.88 (1H, d, *J* = 14.4 Hz, β vinyl *H*), 6.81 (2H, d, *J* = 8.7 Hz, ArH), 6.16 (1H, d, *J* = 14.4 Hz, α vinyl *H*), 1.56 (9H, s, (CH<sub>3</sub>)<sub>3</sub>), 1.43 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 157.4, 136.3, 128.1, 125.8, 120.6, 31.6, 25.6, 22.6, 18.2, 14.1, -4.3

### 4-(methoxymethoxy)phenyl vinyl-isocyanide **580**

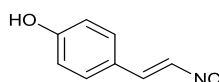


*Compound was originally prepared as a 3:2 mixture of its E/Z isomers using general procedure C with diethyl isocyanomethylphosphonate (PhosMIC) 500. Below we detail using <sup>i</sup>Pr PhosMIC 574 for the E-selective synthesis of this compound, the product of this preparation was used for biological evaluation.*

## Experimental

The title compound was prepared according to general procedure C from LHMDs (1.0 M in THF) (0.4 mL, 0.36 mmol), diisopropyl isocyanomethylphosphonate **574** (74  $\mu$ L, 0.36 mmol) and 4-(methoxymethoxy)benzaldehyde **577** (0.12 mL, 0.0012) in THF (10 mL). Purification was achieved by silica gel chromatography [hexanes : EtOAc (70 : 30),  $R_f$  0.34], affording the title compound as a black oil (28 mg, 49% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.20 (2H, d,  $J$  = 8.6 Hz, ArH), 6.94 (2H, d,  $J$  = 8.6 Hz, ArH), 6.81 (1H, d,  $J$  = 14.3 Hz  $\beta$  vinyl H), 6.09 (1H, d,  $J$  = 14.4 Hz,  $\alpha$  vinyl H), 5.10 (2H, s,  $\text{OCH}_2\text{O}$ ), 3.39 (3H, s,  $\text{OCH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 158.5, 136.1, 128.1 (2C), 116.6 (2C), 94.2, 56.1; IR (film,  $\text{cm}^{-1}$ )  $\nu$  = 2929.29 (C-H aromatic), 2828.18 (C-H aromatic) 2119.28 (IC)

### 4-phenol vinyl-isocyanide **542**<sup>20</sup>



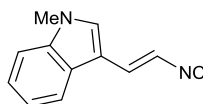
The title compound was prepared according to optimised HWE procedure A using 4-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **576** (200 mg, 0.847 mmol), diisopropyl isocyanomethylphosphonate **574** (208  $\mu$ L, 0.984 mmol) and LHMDs (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.75].  $^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta_{\text{H}}$  = 7.47 (2H, d,  $J$  = 8.5 Hz, ArH), 7.08 (1H, d,  $J$  = 14.3 Hz  $\beta$  vinyl H), 6.92 (2H, d,  $J$  = 8.53, ArH), 6.63 (1H, d,  $J$  = 14.4 Hz,  $\alpha$  vinyl H);  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ ):  $\delta_{\text{C}}$  = 165.8, 160.0, 137.3, 129.6, 125.2, 116.7, 109.4; IR (film,  $\text{cm}^{-1}$ )  $\nu$  = 3241.99 (O-H), 2925.49 (C-H aromatic), 2144.94 (N-C)

## 6.3 Biological evaluation of phenol vinyl-isocyanide (Chapter 3).

### *N*-methyl-3-indole vinyl-isocyanide **553**

Compound Prepared by Liam Stephens.

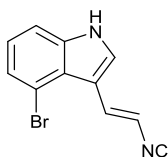
## Experimental



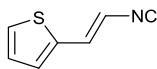
The title compound was prepared according to optimised HWE procedure C using *N*-methyl-indole-3-carbaldehyde (100 mg, 0.629 mmol), diisopropyl isocyanomethylphosphonate **574** (181  $\mu$ L, 0.725 mmol) and LHMDS (1.0 M in THF) (0.8 mL, 0.81 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.35], affording the title compound as a pale yellow oil (45mg, 39% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.75-7.25 (5H, m, ArH), 7.17 (1H, d,  $J$  = 14.3 Hz,  $\beta$  vinyl H), 6.35 (1H, d,  $J$  = 14.3 Hz,  $\alpha$  vinyl H), 3.86 (3H, s,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 162.9, 137.1, 130.7, 130.0, 125.2, 122.9, 121.1, 119.9, 110.0, 109.4, 160.2, 33.0; IR (film,  $\text{cm}^{-1}$ )  $\nu$  = 3051.80 (C-H aromatic), 2929.57 (C-H aromatic), 2116.00 (IC)

### (*E*)-4-bromo-3-(2-isocyanovinyl)-1H-indole **554**

Compound Prepared by Liam Stephens.



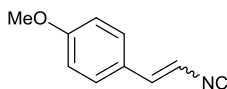
The title compound was prepared according to optimised HWE procedure B using 4-bromo-1H-indole-3-carbaldehyde (100 mg, 0.448 mmol), diisopropyl isocyanomethylphosphonate **574** (110  $\mu$ L, 0.538 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 0.991 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.51], affording the target compound as a pale brown oil (35 mg, 46% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.45 (1H, br s, NH), 7.95 (1H, d,  $J$  = 14.3 Hz,  $\beta$  vinyl H), 7.42 (1H, d,  $J$  = 2.6 Hz, ArH), 7.37 (1H, d,  $J$  = 2.6 Hz, ArH), 7.34 (1H, d,  $J$  = 3.0 Hz), 7.05 (1H, t,  $J$  = 7.9 Hz, ArH), 6.07 (1H, d,  $J$  = 14.3 Hz,  $\alpha$  vinyl H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 132.0, 128.5, 126.8, 126.4, 124.1, 123.2, 120.9, 118.0, 114.4, 110.1; IR (film,  $\text{cm}^{-1}$ )  $\nu$  = 3658.2 (C-H aromatic), 2979.4 (C-H aromatic), 2139.04 (IC)

**(E)-(isocyanovinyl)-2-thiophene 555**

The title compound was prepared according to optimised HWE procedure B using thiophene-2-carboxaldehyde (100 mg, 0.820 mmol), diisopropyl isocyanomethylphosphonate **574** (200  $\mu$ L, 0.984 mmol) and LHMDS (1.0 M in THF) (1.8 mL, 1.8 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (99 : 1),  $R_f$  0.20], affording the title compound as a brown oil (54 mg, 52% yield).  $^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta_H$  = 7.35 (1H, s, ArH), 7.17 (1H, s, ArH), 7.08 (1H, s, ArH), 7.12 (1H, d,  $J$  = 14.2 Hz,  $\beta$  vinyl H), 6.22 (1H, d,  $J$  = 14.2 Hz,  $\alpha$  vinyl H);  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ )  $\delta_C$  = 160.7 (IC), 129.8, 129.3, 128.0, 127.1, 116.4, 109.3; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 2976.09 (C-H aromatic), 2857.55 (C-H aromatic), 2120.67 (IC)

**4-(methoxy)phenyl vinyl-isocyanide 585**

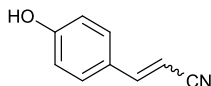
*Compound Prepared by Liam Stephens.*



The title compound was prepared according to optimised HWE procedure C using 4-methoxybenzaldehyde **584** (100 mg, 0.735 mmol), diisopropyl isocyanomethylphosphonate **574** (180  $\mu$ L, 0.822 mmol) and LHMDS (1.0 M in THF) (0.8 mL, 0.81 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.15], affording the title compound as a pale yellow oil (28mg, 24% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_H$  = 7.20 (2H, d,  $J$  = 8.8 Hz, ArH), 6.85 (1H, d,  $J$  = 14.0 Hz,  $\beta$  vinyl H), 6.80 (2H, d,  $J$  = 8.8 Hz, ArH), 6.10 (1H, d,  $J$  = 14.0 Hz,  $\alpha$  vinyl H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_C$  = 160.6, 136.2, 131.5, 131.0, 128.2, 114.5, 114.1, 55.3; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 2960.46 (C-H aromatic), 2838.26 (C-H aromatic), 2118.44 (IC).

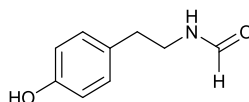
### Phenol vinyl-cyanide **587**

Compound Prepared by Liam Stephens.

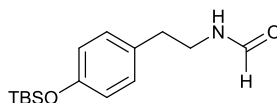


The title compound was prepared according to optimised HWE procedure A using 4-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **560** (200 mg, 0.847 mmol), diethyl cyanomethylphosphonate **586** (208  $\mu$ L, 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude products was purified using silica gel chromatography [hexane : EtOAc (80 : 20),  $R_f$  0.23], affording the title compound as a pale yellow oil (32mg, 28% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.42 (2H, d,  $J$  = 8.8 Hz, ArH), 7.40 (1H, d,  $J$  = 16.6 Hz,  $\beta$  vinyl H), 6.80 (2H, d,  $J$  = 8.8 Hz, ArH), 5.95 (1H, d,  $J$  = 16.6 Hz,  $\alpha$  vinyl H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 162.0, 152.3, 130.7, 127.0, 120.3, 117.0, 93.0; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3279.81 (O-H), 2220.42 ( $\text{C}\equiv\text{N}$ ).

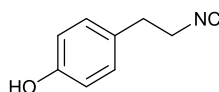
### *N*-(4-hydroxyphenethyl)formamide **589**<sup>211</sup>



Tyramine **588** (200 mg, 1.45 mmol) was dissolved in THF (5 mL) and cooled to 0 °C and acetic formic anhydride (141 mg, 1.60 mmol) added dropwise. After stirring for 2 h., the reaction mixture was concentrated to yield crude target material as pale orange oil, which was used in the next step without further purification (240mg, nr quant. yield).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ):  $\delta_{\text{H}}$  = 9.91 (1H, s, CHO), 7.99 (2H, br s, NH + ArOH), 7.01 (2H, d,  $J$  = 8.3, ArH), 6.67(2H, d,  $J$  = 8.3, ArH), 3.26 (2H, app q,  $J$  = 6.8 Hz,  $\text{NHCH}_2$ ), 2.61 (2H, t,  $J$  = 7.2 Hz,  $\text{NCH}_2\text{CH}_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ ):  $\delta_{\text{C}}$  = 161.3, 156.0, 129.8, 129.6, 115.4, 39.8, 34.5; IR (powder,  $\text{cm}^{-1}$ ):  $\nu$  = 1648.55 ( $\text{C=O}$ ); HRMS (ESI) calculated for  $\text{C}_9\text{H}_{12}\text{NO}_2$   $[\text{M}+\text{H}]^+$ : found  $m/z$  166.0869, requires  $m/z$  166.0868.

***N*-(4-((*tert*-butyldimethylsilyl)oxy)phenethyl)formamide 590**

The title compound was prepared according to general procedure 3 using *N*-(4-hydroxyphenethyl)formamide **589** (0.89 g, 0.0054 mol), TBSCl (0.98 g, 0.0065 mol) and imidazole (1.10 g, 0.0162 mol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The crude reaction residue was purified by silica gel chromatography [pentanes, R<sub>f</sub> 0.61] to afford **590** as pale yellow oil (0.98g, nr quant. yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 8.04 (1H, s, CHO), 7.02 (2H, d, *J* = 8.6, ArH), 6.75 (2H, d, *J* = 8.6, ArH), 3.46 (2H, app q, *J* = 6.8 Hz, NHCH<sub>2</sub>), 2.73 (2H, t, *J* = 6.8 Hz, NCH<sub>2</sub>CH<sub>2</sub>); 0.96 (9H, s, Si(CH<sub>3</sub>)<sub>3</sub>), 0.16 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 161.4 154.2, 131.2, 129.6, 120.1, 39.4, 34.6, 25.7, 18.1, -4.4; IR (film, cm<sup>-1</sup>): ν = 3289.88 (N-H), 2954.90 (C-H aromatic), 2929.82 (C-H aromatic), 2885.28 (C-H aromatic), 2857.88 (C-H aromatic), 1664.02 (C=O); HRMS (ESI) calculated for C<sub>15</sub>H<sub>26</sub>NO<sub>2</sub>Si [M+H]<sup>+</sup>: found *m/z* 280.1733, requires *m/z* 280.1732.

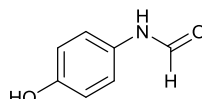
**4-(2-isocyanoethyl)phenol 591**

The title compound was prepared by first dehydrating *N*-(4-((*tert*-butyldimethylsilyl)oxy)phenethyl)formamide **590** (0.98 g, 0.0035 mol) according to general procedure 2, using MsCl (0.82 mL, 0.0107 mol) and Et<sub>3</sub>N (4.48 mL, 0.0322 mol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) to yield the crude silyl protected phenol-isocyanide as a brown oil that was then dissolved in ethanol (15 mL) and treated with KOH (0.5 g approx.). After stirring for 2 h. at r.t., the crude reaction evaporation residue was partitioned between EtOAc and H<sub>2</sub>O, the organics dried over MgSO<sub>4</sub> and then concentrated to give a pale brown oil that was purified by silica gel chromatography [pentanes : EtOAc (50:50), R<sub>f</sub> 0.55] to afford **592** as pale yellow oil (0.42g, 82% yield). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ<sub>H</sub> = 9.93 (1H, br s, OH), 7.07 (2H, d, *J* = 8.67 Hz, ArH),

## Experimental

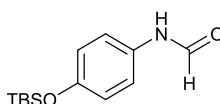
6.72 (2H, d,  $J = 8.67$  Hz, ArH), 3.65 (2H, t,  $J = 6.78$  Hz, NCH<sub>2</sub>), 2.77 (2H, t,  $J = 6.78$  Hz, NCHCH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta_c = 165.5, 155.9$  (IC), 130.1, 127.8, 115.5, 43.3, 43.3, 34.2; IR (film, cm<sup>-1</sup>):  $\nu = 2132.12$  (IC)

### ***N*-(4-Hydroxyphenyl)formamide 593**



The title compound was prepared according general procedure 4 by dissolving 4-aminophenol **592** (1.00 g, 0.0091 mol) in THF (10 mL) and reacting with acetic formic anhydride (0.89 g, 0.0101 mol). The resultant white solid was used in the next step without further purification (1.05 g, nr quant. yield). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>):  $\delta_H = 9.90$  (1H, br s, CHO), 9.26 (1H, br s, OH), 8.14 (1H, br s, NH), 8.49 (0.3H, d,  $J = 9.0$  Hz, ArH (Z rotamer)), 7.36 (1.7H, d,  $J = 9.0$  Hz, ArH (E rotamer)), 6.97 (0.3H, d,  $J = 9.0$  Hz, ArH (Z rotamer)), 6.68 (1.7H, d,  $J = 9.0$  Hz, ArH (E rotamer)); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta_c = 162.9$  (Z rotamer), 159.2 (E rotamer), 154.5 (Z rotamer), 153.8 (E rotamer), 130.3 (E rotamer), 129.9 (Z rotamer), 121.1 (E rotamer), 120.5 (Z rotamer), 116.1 (Z rotamer), 115.5 (E rotamer); IR (film, cm<sup>-1</sup>):  $\nu = 3303.39$  (N-H), 2978.48 (C-H aromatic), 2889.61 (C-H aromatic), 1657.48 (C=O); HRMS (ESI) calculated for C<sub>7</sub>H<sub>8</sub>NO<sub>2</sub> [M+H]<sup>+</sup>: found  $m/z$  138.0568, requires  $m/z$  138.0555.

### ***N*-(4-((tert-butyldimethylsilyl)oxy)phenyl)formamide 594**

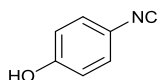


The title compound was prepared according to general procedure 3 using *N*-(4-hydroxyphenyl)formamide **593** (585 mg, 4.27 mmol), TBSCl (768 mg, 768 mmol) and imidazole (800 mg, 12.81 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL). The crude reaction residue was purified by silica gel

## Experimental

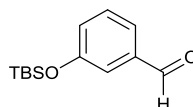
chromatography [pentanes : EtOAc (80 : 20),  $R_f$  0.15] to afford **594** as pale yellow oil (982 mg, 91% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.52 (0.5H, d,  $J$  = 11.6 Hz, CHO (rotamer)), 8.34 (0.5H, d,  $J$  = 1.8 Hz, CHO (rotamer)), 7.55 (0.5H, br s, NH (rotamer)), 7.39 (1H, d,  $J$  = 9.0 Hz, ArH (rotamer)), 7.25 (0.5H, br s, NH (rotamer)), 6.97 (1H, d,  $J$  = 9.0 Hz, ArH (rotamer)), 6.85 (1H, d,  $J$  = 6.0 Hz, ArH (rotamer)), 6.82 (1H, d,  $J$  = 6.0 Hz, ArH (rotamer));  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 162.8, 158.7, 121.5, 121.1, 120.5, 25.6, 18.2, -4.4; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3268.98 (N-H), 2958.75 (C-H aromatic), 2930.62 (C-H aromatic), 2859.05 (C-H aromatic), 1672.20 (C=O); HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{21}\text{NO}_2\text{Si}$   $[\text{M}+\text{H}]^+$ : found  $m/z$  252.1418, requires  $m/z$  252.1419.

### 4-isocyanophenol **595**

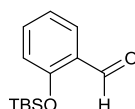


The title compound was prepared by first dehydrating *N*-(4-((tert-butyldimethylsilyl)oxy)phenyl)formamide **594** (550 mg, 2.19 mmol) according to general procedure 2, using  $\text{MsCl}$  (0.50 mL, 0.0107 mol) and  $\text{Et}_3\text{N}$  (2.68 mL, 19.71 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) to yield the crude *O*-silyl protected phenol-isocyanide as a brown oil that was then dissolved in ethanol (15 mL) and treated with  $\text{KOH}$  (0.5 g approx.). After stirring for 2 h. at r.t., the crude reaction evaporation residue was partitioned between EtOAc and  $\text{H}_2\text{O}$ , the organics dried over  $\text{MgSO}_4$  and then concentrated to give a pale brown oil that was purified by silica gel chromatography [pentanes : EtOAc (80 : 20),  $R_f$  0.45] to afford **595** as pale yellow oil (180 mg, 69% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.26 (2H, d,  $J$  = 8.6 Hz, ArH), 6.85 (2H, d,  $J$  = 8.6 Hz, ArH);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 160.4, 157.0, 128.0 (2C), 116.2; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3382.31 (O-H), 2908.25 (C-H aromatic), 2947.60 (C-H aromatic), 2124.90 (IC).



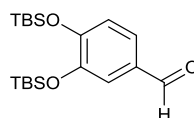
**3-((tert-butyldimethylsilyl)oxy)benzaldehyde 599**<sup>212</sup>

The title compound was prepared according to general procedure 2 using *m*-hydroxybenzaldehyde **596** (5.00 g, 0.041 mol), imidazole (3.10 g, 0.045 mol), TBSCl (7.35 g, 0.045 mol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 9.92 (1H, s, CHO), 7.45 (1H, dt, *J* = 7.6, 1.4 Hz, ArH), 7.36 (1H, t, *J* = 7.8 Hz, ArH), 7.31 (1H, dd, *J* = 2.5, 1.4 Hz, ArH), 7.07 (1H, ddd, *J* = 7.8, 2.5, 1.4 Hz, ArH), 0.97 (9H, s, Si(CH<sub>3</sub>)<sub>3</sub>), 0.19 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 192.2, 156.4, 137.8, 130.1, 126.6, 23.6, 119.8, 25.6, -4.4; IR (film, cm<sup>-1</sup>): ν = 1701.16 (C=O); HRMS (ESI) calcd for C<sub>13</sub>H<sub>20</sub>NaO<sub>2</sub>Si [M+Na]<sup>+</sup>: found *m/z* 259.1113, requires *m/z* 259.1130.

**2-((tert-butyldimethylsilyl)oxy)benzaldehyde 600**<sup>213</sup>

The title compound was prepared according to general procedure 2 using 2-hydroxybenzaldehyde **597** (5.00 g, 0.041 mol), imidazole (3.10 g, 0.045 mol) and TBSCl (7.35 g, 0.045 mol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 10.44 (1H, s, CHO), 7.78 (1H, d, *J* = 7.8 Hz, ArH), 7.41 (1H, t, *J* = 7.7 Hz, ArH), 6.98 (1H, t, *J* = 7.8 Hz, ArH), 6.85 (1H, d, *J* = 7.7 Hz, ArH), 0.98 (9H, s, Si(CH<sub>3</sub>)<sub>3</sub>), 0.24 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 189.9, 158.8, 135.6, 128.2, 127.4, 121.7, 121.0, 25.6, 18.3, -4.3; IR (film, cm<sup>-1</sup>): ν = 1687.60 (C=O); HRMS (ESI) calculated for C<sub>13</sub>H<sub>20</sub>NaO<sub>2</sub>Si [M+Na]<sup>+</sup>: found *m/z* 259.1213, requires *m/z* 259.1330.

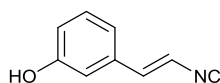
### 3,4-bis-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **601**



The title compound was prepared according to general procedure 2 using 3,4-dihydroxybenzaldehyde **598** (0.5 g, 3.62 mmol), imidazole (1.37 g, 21.72 mmol) and TBSCl (1.69 g, 10.86 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). The crude products were purified by silica gel chromatography [pentanes : EtOAc (95 : 5), R<sub>f</sub> 0.90], affording the title compound as colourless oil (1.13 g, 86% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 9.82 (1H, s, CHO), 7.38 (1H, d, *J* = 8.0 Hz, ArH), 7.36 (1H, s, ArH), 6.95 (1H, d, *J* = 8.2 Hz, ArH), 1.01 (9H, s, SiC(CH<sub>3</sub>)<sub>3</sub>), 1.00 (9H, s, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.26 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>), 0.24 (6H, s, Si(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 190.8, 153.3, 147.6, 130.7, 125.2, 120.8, 25.8, 18.4, -4.1; IR (film, cm<sup>-1</sup>): ν = 2955.67 (C-H aromatic), 2930.85 (C-H aromatic), 2859.16 (C-H aromatic), 1694.53 (C=O); HRMS (ESI) calculated for C<sub>19</sub>H<sub>34</sub>NaO<sub>3</sub>Si<sub>2</sub> [M+Na]<sup>+</sup>: found *m/z* 389.1955, requires *m/z* 389.1944.

### 3-phenol vinyl-isocyanide **602**

Compound prepared by Liam Stephens



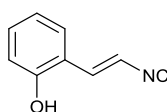
The title compound was prepared according to optimised HWE procedure A using 3-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **599** (200 mg, 0.847 mmol), diisopropyl isocyanomethylphosphonate **574** (208 μL, 0.984 mmol) and LHMDs (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), R<sub>f</sub> 0.63]. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 7.30-7.20 (1H, m, ArH), 6.95 – 6.80 (3H, m, ArH), 6.90 (1H, d, *J* = 14.1 Hz, β

## Experimental

vinyl *H*), 6.25 (1H, d,  $J = 14.1$  Hz,  $\alpha$  vinyl *H*);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}} = 157.1, 134.4, 131.9, 130.5, 121.1, 117.2, 116.8, 79.6$ ; IR (film,  $\text{cm}^{-1}$ ):  $\nu = 3272.77$  (O-H), 2924.35 (C-H aromatic), 2853.09 (C-H aromatic), 2111.63 (IC)

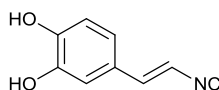
*This species rapidly isomerised to a mixture of E/Z isomers*

### 2-phenol vinyl-isocyanide **603**



The title compound was prepared according to optimised HWE procedure A using 2-((*tert*-butyldimethylsilyl)oxy)benzaldehyde **600** (200 mg, 0.847 mmol), diisopropyl isocyanomethylphosphonate **574** (208  $\mu\text{L}$ , 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.20] to afford title compound as a pale yellow oil (21 mg, 25% yield).  $^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta_{\text{H}} = 7.58$  (1H, br d,  $J = 7.3$  Hz, OH), 7.27 (1H, dd,  $J = 7.7, 1.6$  Hz, ArH), 7.12-7.04 (1H, m, ArH), 6.99 (1H, d,  $J = 14.1$  Hz,  $\beta$  vinyl *H*), 6.85 (1H, d,  $J = 8.5$  Hz, ArH), 6.77-6.66 (2H, m, ArH +  $\alpha$  vinyl *H*);  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ ):  $\delta_{\text{C}} = 157.1, 134.4, 131.9, 130.5, 121.1, 117.2, 116.8, 79.6$ ; IR (film,  $\text{cm}^{-1}$ ):  $\nu = 3361.05$  (O-H), 2976.09 (C-H aromatic), 2857.55 (C-H aromatic), 2116.04 (IC)

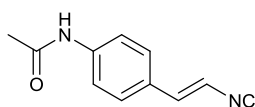
### 3,4-bis-phenol vinyl-isocyanide **557**



## Experimental

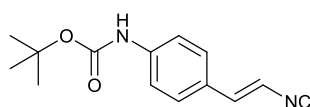
The title compound was prepared according to optimised HWE procedure A using 3,4-bis-((tert-butyldimethylsilyl)oxy)benzaldehyde **601** (100 mg, 0.27 mmol), diisopropyl (isocyanomethyl) phosphonate **574** (66  $\mu$ L, 0.984 mmol) and LHMDS (1.0 M in THF) (0.3 mL, 0.32 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexane : EtOAc (80 : 20),  $R_f$  0.05] to afford title compound as a pale yellow oil (15 mg, 20% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta_{\text{H}}$  = 6.81-6.73 (2H, m, *ArH*), 6.70-6.60 (2H, m, *ArH* +  $\beta$  vinyl *H*), 6.25 (1H, d,  $J$  = 14.32 Hz,  $\alpha$  vinyl *H*);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta_{\text{C}}$  = 149.1, 147.2, 138.8, 126.5, 124.0, 121.4, 116.9, 114.5; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3220.54 (O-H), 2982.63 (C-H aromatic), 2933.75 (C-H aromatic), 2119.57 (IC)

### (*E*)-(isocyanovinyl)-*N*-(4-formylphenyl) acetamide **607**



The title compound was prepared according to optimised HWE procedure B using *N*-(4-formylphenyl)acetamide **604** (100 mg, 0.613 mmol), diisopropyl isocyanomethylphosphonate **574** (120  $\mu$ L, 0.736 mmol) and LHMDS (1.0 M in THF) (3.00 mL, 3.03 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (50 : 50),  $R_f$  0.20], affording the title compound as a brown oil (42 mg, 35% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.54 (2H, d,  $J$  = 8.7 Hz, *ArH*), 7.31 (2H, d,  $J$  = 8.7 Hz, *ArH*), 6.90 (1H, d,  $J$  = 14.3 Hz,  $\beta$  vinyl *H*), 6.23 (1H, d,  $J$  = 14.3 Hz,  $\alpha$  vinyl *H*), 2.20 (3H, s,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 168.5, 165.0, 139.8, 136.2, 128.6, 127.2, 119.7, 109.5, 24.9; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3253.59 (N-H), 3183.68 (C-H aromatic), 3114.16 (C-H aromatic), 3071.34 (C-H aromatic), 2116.77 (IC), 1667.06 (C=O).

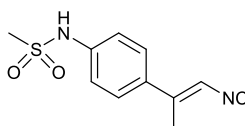
### (*E*)-(isocyanovinyl)-*tert*-butyl-4-formylphenyl **608**



## Experimental

The title compound was prepared according to optimised HWE procedure B using *tert*-butyl-4-formylphenylcarbamate **605** (100 mg, 0.488 mmol), diisopropyl isocyanomethylphosphonate **574** (120  $\mu$ L, 0.585 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.0 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (95 : 5),  $R_f$  0.30], affording the title compound as a brown oil (38 mg, 31% yield).  $^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta_H$  = 7.14 (2H, d,  $J$  = 9.0 Hz, ArH), 7.04 (2H, d,  $J$  = 9.0, ArH), 6.65 (1H, d,  $J$  = 14.3 Hz,  $\beta$  vinyl H), 5.97 (1H, d,  $J$  = 14.3 Hz,  $\alpha$  vinyl H), 1.29 (9H, s,  $(\text{CH}_3)_3$ );  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ ):  $\delta_C$  = 160.7 (IC), 153.9, 142.3, 137.4, 128.7, 119.6, 112.5, 81.1, 29.2; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 2976.09 (C-H aromatic), 2857.55 (C-H aromatic), 2123.08 (IC)

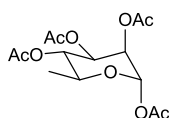
### (*E*)-*N*-(4-(1-isocyanoprop-1-en-2-yl)phenyl)methanesulfonamide **609**



The title compound was prepared according to optimised HWE procedure B using *N*-(4-acetylphenyl)-methanesulfonamide **604** (50 mg, 0.302 mmol), diisopropyl isocyanomethylphosphonate **574** (61  $\mu$ L, 0.302 mmol) and LHMDS (1.0 M in THF) (0.6 mL, 0.55 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20),  $R_f$  0.12 ], affording the title compound as a yellow oil (12 mg, 16% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_H$  = 7.33 (2H, d,  $J$  = 9.0 Hz, ArH), 7.22 (2H,  $J$  = 9.0 Hz, ArH), 5.99 (1H, s, vinyl CH), 3.01 (3H, s,  $\text{SCH}_3$ ), 2.24 (3H, s,  $\text{CCH}_3$ );  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_C$  = 142.9, 137.9, 128.9, 127.2, 120.2, 39.6, 16.8. IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3249.99 (N-H) 3024.61 (C-H aromatic), 2983.74, (C-H aromatic), 2930.51 (C-H aromatic), 2114.18 (IC)

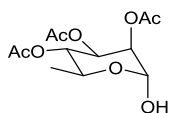
#### 6.4 Compounds associated with the synthesis of Byelyankacin and Rhabduscin (Chapter 4).

##### Per-*O*-acetyl-6-deoxy- $\alpha$ -L-mannopyranose **611**<sup>214</sup>



The title compound was prepared according to general procedure 5 from L-Rhamnose **610** (3.00 g, 0.016 mol), DMAP (0.20 g, 10 mol%) and Ac<sub>2</sub>O (30 mL) in pyridine (30 mL). The title compound, a colourless glass, was used in the subsequent reactions without the need for purification (4.92 g, 89% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  = 6.03 (1H, br d,  $J$  = 1.9 Hz, CH-1), 5.32 (1H, dd,  $J$  = 10.1, 3.6 Hz, CH-3), 5.26 (1H, m, CH-2), 5.14 (1H, t,  $J$  = 10.1 Hz CH-4), 3.95 (1H, m, CH-5), 2.18 (3H, s, OAc), 2.17 (3H, s, OAc), 2.08 (3H, s, OAc), 2.02 (3H, s, OAc) 1.25 (3H, d,  $J$  = 6.2 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  = 170.1, 169.8, 169.8, 168.4, 90.6, 70.5, 68.7, 68.7, 68.6, 20.9, 20.8, 20.8, 20.7, 17.4; IR (film, cm<sup>-1</sup>):  $\nu$  = 1742.49 (C=O); HRMS (ESI) calcd for C<sub>14</sub>H<sub>20</sub>NaO<sub>9</sub> [M+Na]<sup>+</sup>: found  $m/z$  355.1014, requires  $m/z$  355.1005.

##### 2,3,4-Tri-*O*-acetyl-6-deoxy- $\alpha$ -L-mannopyranose **614**<sup>215</sup>

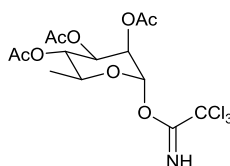


Benzylamine (0.25 mL, 2.25 mmol) was added dropwise to a solution of Per-*O*-acetyl-6-deoxy- $\alpha$ -L-mannopyranose **611** (0.50 g, 1.51 mmol) in THF (10 mL), and left to stir under nitrogen for 16 h. After this time, aq. 1M HCl (5 mL) was added, and after stirring for an additional 30 min. the reaction was diluted further with additional aq. 1M HCl (25 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 25 mL). The organics were combined, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to give the desired compound as an amorphous solid that was used in the next step without purification

## Experimental

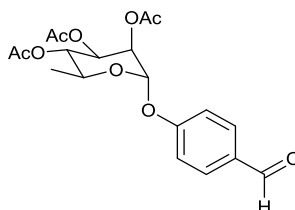
(0.31 g, 70% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 5.28 (1H, dd,  $J$  = 10.1, 3.4 Hz, CH-3), 5.18 (1H, app s, CH-1), 5.08 (1H, s, OH), 4.99 (1H, t,  $J$  = 10.1 Hz, CH-4), 4.33 (1H, d,  $J$  = 4.1 Hz, CH-2) 4.07 (1H, app m, CH-5), 2.09 (3H, s, OAc), 2.00 (3H, s, OAc), 1.93 (3H, s, OAc) 1.25 (3H, d,  $J$  = 6.2 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 170.4, 170.3, 170.2, 92.5, 91.9, 70.4, 68.6, 20.8, 20.7, 20.6, 17.4; IR (powder,  $\text{cm}^{-1}$ ):  $\nu$  = 3421.10 (O-H), 1742.65 (C=O), 1721.27 (C=O); HRMS (ESI) calcd for  $\text{C}_{12}\text{H}_{18}\text{NaO}_8$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  313.0894, requires  $m/z$  313.0870

### (2,3,4-Tri-O-acetyl- $\alpha$ -rhamnopyranosyloxy)-trichloroacetimidate **615**



(2R,3R,4R,5S,6S)-2-hydroxy-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate **614** (0.251 g, 0.86 mmol), trichloroacetonitrile (0.17 mL, 1.72 mmol) and  $\text{CsCO}_3$  (0.140 g, 0.43 mmol) were dissolved/suspended in  $\text{CH}_2\text{Cl}_2$  (10 mL) and stirred under nitrogen for 2.5 h. The reaction mixture was then filtered through a short pad of silica gel, which was subsequently washed with a mixed solution of petroleum ether : EtOAc (50 : 50) (200 mL). The combined filtrates were then concentrated *in vacuo* to give the title compound as a pale yellow oil (0.368 g, 99% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.72 (1H, s, NH), 6.19 (1H, d,  $J$  = 2.0 Hz, CH-1), 5.45 (1H, dd,  $J$  = 3.5, 2.0 Hz, CH-2), 5.35 (1H, dd,  $J$  = 10.3, 3.5 Hz, CH-3), 5.16 (1H, t,  $J$  = 10.3 Hz, CH-4), 4.08 (1H, app m, CH-5), 2.18 (3H, s, OAc), 2.06 (3H, s, OAc), 2.00 (3H, s, OAc) 1.31, (3H, d,  $J$  = 6.2 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 169.9, 169.9, 169.8, 160.0, 94.6, 90.7, 70.3, 69.3, 68.8, 68.2, 20.8, 20.8, 20.7, 17.5; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 1731.42 (C=NH), 1721.27 (C=O); HRMS (ESI) calcd for  $\text{C}_{14}\text{H}_{18}\text{Cl}_3\text{NNaO}_8$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  456.0007, requires  $m/z$  455.9995

**4-(2,3,4-Tri-O-acetyl- $\alpha$ -rhamnopyranosyloxy)-benzaldehyde **613**<sup>194</sup>**



*First preparation (from trichloroacetimidate **615**)*

(2,3,4-Tri-O-acetyl- $\alpha$ -rhamnopyranosyloxy)-trichloroacetimidate **615** (0.36 g, 0.83 mmol) and 4-hydroxybenzaldehyde **560** (0.10 g, 0.83 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL), and TMS.OTf (0.15 mL, 0.83 mmol) added dropwise over 30 min. The resulting mixture was stirred for 48 h., before aq.  $\text{NaHCO}_3$  was added and the whole stirred for an additional 5 min. Following extraction with  $\text{CH}_2\text{Cl}_2$  (2 x 50 mL), the organics were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to afford a pale yellow oil. Purification was achieved by flash column chromatography [petroleum ether : EtOAc (50 : 50),  $R_f$  0.33] to furnish title compound as a pale yellow oil (99 mg, 20% yield).

*Second Preparation (from thioether **616** - next compound)*

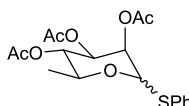
The title compound was prepared according to general procedure 7 from the addition of triflic acid (124  $\mu\text{L}$ , 0.0014 mol) and *N*-iodosuccinamide (1.3 g, 0.0058 mol) to a solution of thiophenyl-2,3,4-Tri-O-acetyl-L-rhamnopyranoside **616** (2.25 g, 0.0058 mol) and 4-hydroxybenzaldehyde **560** (0.70 g, 0.0058) in  $\text{CH}_2\text{Cl}_2$ . The evaporation residue was purified by silica gel chromatography [petroleum ether : EtOAc (70 : 30),  $R_f$  0.33] yielding a pale yellow oil (1.38 g, 60% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 9.90 (1H, s, CHO), 7.85 (2H, d,  $J$  = 8.7 Hz, ArH), 7.19 (2H, d,  $J$  = 8.7 Hz, ArH), 5.55 (1H, d,  $J$  = 1.8 Hz, CH-1), 5.49 (1H, dd,  $J$  = 9.9, 3.5 Hz, CH-4), 5.43 (1H, m, CH-3), 5.39 (1H, t,  $J$  = 9.9 Hz, CH-2), 3.91 (1H, dq, CH-5), 2.19 (3H, s, OAc), 2.04 (3H, s, OAc), 2.02 (3H, s, OAc) 1.19 (3H, d,  $J$  = Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 190.7, 170.0, 160.4, 131.9, 131.4, 116.4, 95.3, 70.6, 69.3, 68.6, 67.6, 20.7, 17.4; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  =



## Experimental

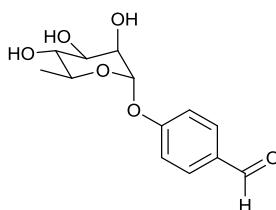
2982.26 (C-H aromatic), 1746.54 (C=O *ester*), 1691 (C=O *aldehyde*); HRMS (ESI) calculated for  $C_{19}H_{23}O_9$   $[M+H]^+$ : found  $m/z$  395.1335, requires  $m/z$  395.1342.

### Thiophenyl-2,3,4-Tri-O-acetyl-L-rhamnopyranoside **616**<sup>216</sup>



The title compound was prepared according to general procedure 7 from the addition of boron trifluoro ethyl etherate (2.1 mL, 0.017 mol) to per-*O*-acetyl-6-deoxy- $\alpha$ -L-mannopyranose **611** (5.80 g, 0.017 mol) and thiophenol (1.68 mL, 0.017 mol) dissolved in toluene (30 mL). The crude evaporation residue was purified by silica gel chromatography [petroleum ether : EtOAc (70 : 30),  $R_f$  0.30] to afford a pale yellow solid (5.12 g, 78 % yield).  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta_H$  = 7.40-7.49 (2H, m, *ArH*), 7.21-7.30 (3H, m, *ArH*), 5.49 (0.33H, d,  $J$  = 3.2 Hz,  $CH-2_\beta$ ), 5.44 (0.66H, d,  $J$  = 3.3 Hz,  $CH-2_\alpha$ ) 5.35 (0.66H, app s,  $CH-1_\alpha$ ) 5.28 (0.66H, dd,  $J$  = 10.2, 3.2 Hz,  $CH-3_\alpha$ ), 5.11-5.03 (1H, app m,  $CH-4$ ), 4.95 (0.33H, dd,  $J$  = 10.2, 3.3,  $CH-3_\beta$ ), 4.84 (0.33H, app s,  $CH-1_\beta$ ), 4.30 (0.66H, dq,  $J$  = 10.2, 6.0 Hz,  $CH-5_\alpha$ ), 3.49 (0.33H, dq,  $J$  = 10.2, 6.0 Hz,  $CH-5_\beta$ ), 2.14 (1H, s,  $OAc_\beta$ ), 2.08 (2H, s,  $OAc_\alpha$ ), 2.01 (2H, s,  $OAc_\alpha$ ), 1.98 (1H, s  $OAc_\beta$ ), 1.95 (2H, s,  $OAc_\alpha$ ), 1.92 (1H, s,  $OAc_\beta$ ), 1.25 (1H, d,  $J$  = 6.0 Hz,  $CH_{3\beta}$ ), 1.18 (2H, d,  $J$  = 6.0 Hz,  $CH_{3\alpha}$ );  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ):  $\delta_C$  = 170.1, 170.0, 169.9, 133.2, 132.0, 131.8, 129.2, 129.1, 128.0, 127.8, 85.6, 85.3, 72.0, 71.8, 71.3, 71.1, 71.0, 70.2, 69.3, 67.7, 20.9, 20.8, 20.7, 20.6, 20.6, 20.6, 17.7, 17.3; IR (powder,  $cm^{-1}$ ):  $\nu$  = 2990.24 (C-H aromatic), 1742.27 (C=O); HRMS (ESI) calculated for  $C_{18}H_{22}NaO_7S$   $[M+Na]^+$ : found  $m/z$  405.1016, requires  $m/z$  405.0983

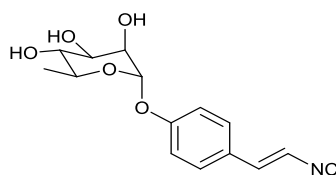
### 4-( $\alpha$ -rhamnopyranosyloxy)-benzaldehyde **617**<sup>194</sup>



## Experimental

The title compound was prepared according to general procedure 8 from a mixture of (2,3,4-tri-*O*-acetyl- $\alpha$ -rhamnopyranosyloxy)-benzaldehyde **613** (500 mg, 1.26 mmol) and NaOMe (0.5 M in MeOH) (0.6 mL, 0.315 mmol) in MeOH (10mL). The crude was purified by silica gel chromatography [petroleum ether : acetone (60 : 40),  $R_f$  0.55] to afford a beige solid (335 mg, quant. yield).  $^1\text{H}$  NMR (500 MHz, acetone- $d_6$ ):  $\delta_H$  = 9.93 (1H, s, CHO), 7.89 (2H, d,  $J$  = 8.8 Hz, ArH), 7.26 (2H, d,  $J$  = 8.8 Hz, ArH), 5.65 (1H, app s, CH-1), 4.31 (1H, app s, CH-2), 4.15 (1H, br s, OH), 4.05 (2H, br s, 2x OH), 3.86 (1H, d,  $J$  = 7.8 Hz, CH-3), 3.62 (1H, dq,  $J$  = 9.1, 5.9 Hz, CH-5), 3.51 (1H, t,  $J$  = 9.1 Hz, CH-4), 1.19 (3H, d,  $J$  = 5.9 Hz, CH);  $^{13}\text{C}$  NMR (125 MHz, acetone- $d_6$ ):  $\delta_C$  = 206.8, 191.7, 162.6, 132.7, 132.4, 117.8, 99.5, 73.7, 72.5, 71.8, 71.0, 18.5 IR (powder,  $\text{cm}^{-1}$ ):  $\nu$  = 3313.98 (O-H), 2935.43 (C-H aromatic), 1746.54 (C=O); HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{16}\text{O}_6$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  291.0823, requires  $m/z$  291.0844.

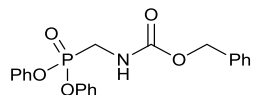
### Byelyankacin **544**<sup>20,196</sup>



4-( $\alpha$ -Rhamnopyranosyloxy)-benzaldehyde **617** (100 mg, 0.37 mmol) was added to a -78 °C solution of diisopropyl isocyanomethylphosphonate **574** (77  $\mu\text{L}$ , 0.37 mmol) and LHMDs (1.0M in THF) (1.67 mL, 1.67 mmol) according to a modified version of general procedure B (4.5 equiv. of base). The title compound was purified using silica gel chromatography [hexane : EtOAc ( 25 : 75 ),  $R_f$  0.35], affording the natural product as pale brown oil (18 mg, 14% yield).  $^1\text{H}$  NMR (500 MHz, acetone- $d_6$ ):  $\delta_H$  = 7.40 (2H, d,  $J$  = 7.8 Hz, ArH), 7.06 (2H, d,  $J$  = 8.9 Hz, ArH), 6.99 (1H, d,  $J$  = 14.5 Hz,  $\beta$  vinyl H), 6.52 (1H, d,  $J$  = 14.5 Hz,  $\alpha$  vinyl H), 5.52 (1H, m, CH-1), 4.32 (1H, s, OH), 4.13, (1H, s, OH), 4.04 (1H, s, OH), 3.98 (1H, m, CH-2), 3.82 (1H, m, CH-3), 3.57 (1H, m, CH-5), 3.45 (1H, m, CH-4), 1.20 (3H, app s,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz, acetone- $d_6$ ):  $\delta_C$  = 164.7 (IC), 159.0, 137.6, 129.5, 128.3, 117.8, 110.6, 99.7, 73.7, 72.2, 71.9, 70.8, 18.0; HRMS (ESI) calculated for  $\text{C}_{15}\text{H}_{17}\text{NNaO}_5$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  314.0983, requires  $m/z$  314.1004;  $\alpha[\text{D}]^{298}$  (MeOH) -105°

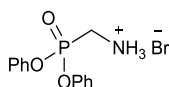
### Synthesis of Bulkier Phosphonates

#### Benzyl ((diphenoxyphosphoryl)methyl)carbamate **627**



A mixture of benzylcarbamate **626** (10.00 g, 0.066 mol), acetic anhydride (9.5 mL, 0.099 mol) and *para*formaldehyde **622** (1.9 g, 0.066 mol) was stirred in acetic acid for 3 h. at 70 °C under nitrogen. Triphenylphosphite (20.0 mL, 0.066 mol) was then added and the reaction temperature increased to 120 °C for 3 h. before the volatiles were removed by rotary evaporation (boiling water bath), and the resulting colourless residue purified by recrystallisation from MeOH, affording the title compound as colourless needles (15.20 g, 57% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.45-7.07 (15H, m, ArH), 5.43 (1H, br s, NH), 5.14 (2H, s,  $\text{CH}_2\text{Ar}$ ), 3.98 (2H, dd,  $J$  = 10.5, 6 Hz,  $\text{PCH}_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 149.9, 129.8, 128.5, 128.3, 125.5, 120.5, 67.4;  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 16.3; IR (film  $\text{cm}^{-1}$ ):  $\nu$  = 1720.15 (C=O); HRMS (ESI) calculated for  $\text{C}_{21}\text{H}_{21}\text{NO}_5\text{P}$   $[\text{M}+\text{H}]^+$ : found  $m/z$  398.1154, requires  $m/z$  398.1157.

#### (Diphenoxyphosphoryl)methanaminium bromide **628**

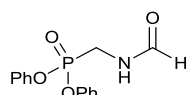


Benzyl ((diphenoxyphosphoryl)methyl)carbamate **627** (7.00 g, 0.017 mol) was dissolved in hydrogen bromide (33% w/w (45% w/v) soln. in acetic acid) (20 mL) and stirred at room temperature for 2 h. After this time, the reaction was concentrated to afford an orange oil, which when triturated with hot diethyl ether precipitated the desired hydro bromide salt as a beige solid (5.90 g, nr. quant. yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.81 (3H, br s,  $\text{NH}_3$ ), 7.50-7.18 (10H, m, ArH), 3.88 (2H, d,  $J$  = 13.6 Hz,  $\text{PCH}_2$ );  $^{13}\text{C}$

## Experimental

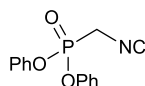
NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 149.6, 130.4, 126.2, 121.0, 33.8 (d,  $J$  = 157.5 Hz);  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{P}}$  = 15.1; HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{15}\text{NO}_3\text{P}$   $[\text{M}]^+$ : found  $m/z$  264.0774, requires  $m/z$  264.0789.

### Diphenyl (formamidomethyl)phosphonate **620**



(Diphenoxyphosphoryl)methanaminium bromide **628** (5.10 g, 0.0147 mol) was dissolved in THF (25 mL), free-based using  $\text{Et}_3\text{N}$  (4.00 mL, 0.0294 mol) and then formylated according to general procedure 4 using acetic formic anhydride (1.42 g, 0.0162). (NOTE: reaction mixture required filtration before removal of volatiles *in vacuo*). The crude products were purified by silica gel chromatography [pentanes : EtOAc (50 : 50),  $R_f$  0.10] to afford title compound as a white solid (4.92 g, near quant. yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.05 (1H, s, CHO), 7.35-7.10 (11H, m, ArH + NH), 4.02 (2H, dd,  $J$  = 11.6, 5.6 Hz,  $\text{PCH}_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 161.3, 149.8, 129.9, 125.8, 120.5, 33.6 (d,  $J$  = 160.7 Hz);  $^{31}\text{P}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{P}}$  = 16.3; IR (powder,  $\text{cm}^{-1}$ ):  $\nu$  = 3290.59 (N-H), 2928.94 (C-H aromatic), 1669.87 (C=O); HRMS (ESI) calculated for  $\text{C}_{14}\text{H}_{15}\text{N}_1\text{O}_4\text{P}_1$   $[\text{M}+\text{H}]^+$ : found  $m/z$  292.0732, requires  $m/z$  292.0738.

### Diphenyl (isocyanomethyl)phosphonate **629**

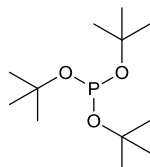


Diphenyl (formamidomethyl)phosphonate **620** (1.00 g, 0.0034 mol) was dehydrated according to general procedure 2 when dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and  $\text{Et}_3\text{N}$  (4.20 mL, 0.030 mol) and treated with  $\text{MsCl}$  (0.77 mL, 0.010 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes : EtOAc (80 : 20),  $R_f$  0.28] to furnish title compound as a

## Experimental

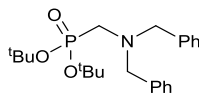
pale orange oil (0.67 g, 72% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.45-7.20 (10H, m, ArH), 4.07 (2H, d,  $J$  = 15.4 Hz,  $\text{PCH}_2$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 162.3, 149.9, 130.1, 126.1, 120.5, 40.4 (d,  $J$  = 159.7 Hz);  $^{31}\text{P}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{P}}$  = 7.16; IR (film  $\text{cm}^{-1}$ ):  $\nu$  = 2135 (IC)

### Tri-*tert*butylphosphite<sup>217</sup>



Phosphorustrichloride (10.00 g, 0.075 mol) in diethyl ether (100 mL) is added dropwise onto a mixture of *tert*-butanol (96.23 mL, 0.225 mol) and  $\text{Et}_3\text{N}$  (61 mL, 0.500 mol) held at 0 °C under nitrogen. The whole is warmed up to r.t. and stirred for 24 h. before being diluted with diethyl ether (100 mL) and filtered. The colourless evaporation residue crystallised upon standing at 4 °C to afford the target material as colourless plates (11.2 g, 68% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 1.30;  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 30.8 (d,  $J$  = 159.7 Hz) 28.6;  $^{31}\text{P}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{P}}$  = 141.0

### Di-*tert*-butyl ((dibenzylamino)methyl)phosphonate **623**

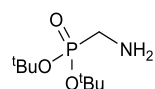


*Para*formaldehyde **622** (0.110 g, 0.0039 mol) was added to dibenzylamine **621** (0.71 mL, 0.0035 mol) dissolved in MeCN (10 mL) and the whole refluxed (bath temp. 80 °C) under nitrogen for 1 h. The resulting suspension was then cooled to r.t. and  $\text{P}(\text{O}^t\text{Bu})_3$  (1.00 g, 0.0039 mol) added dropwise as a solution in MeCN (1 mL) and the whole allowed to stir for 16 h. before the volatiles were removed by rotary evaporation. The evaporation residue was dissolved in EtOAc, washed with 1M HCl, water and brine before being dried over  $\text{MgSO}_4$  and

## Experimental

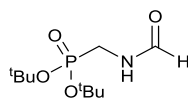
concentrated to yield a pale yellow oil that was purified by silica gel chromatography [pentanes : EtOAc (80 : 20),  $R_f$  0.41] to furnish title compound as a colourless oil (1.24 g, 84% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  7.45-7.17 (10H, m, ArH), 3.88 (2H, s,  $\text{CH}_2\text{Ph}$ ), 2.75 (2H, d,  $J$  = 10.2 Hz,  $\text{PCH}_2$ ), 1.47 (18H, s,  $\text{O}(\text{CH}_3)_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  139.2, 129.0, 128.1, 126.9, 81.8 (d,  $J$  = 157.5 Hz), 59.0, 30.5, 29.9;  $^{31}\text{P}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{P}}$  19.16; HRMS (ESI) calculated for  $\text{C}_{23}\text{H}_{34}\text{NO}_3\text{P}$   $[\text{M}+\text{H}]^+$ : found  $m/z$  404.2352, requires  $m/z$  404.2354.

### Di-tert-butyl (aminomethyl)phosphonate **624**



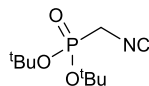
Di-tert-butyl ((dibenzylamino)methyl)phosphonate **623** (660 mg, 1.63 mmol) was dissolved in MeOH (10 mL), Pd/C (10% wt.) (174 mg, 10 mol%) added and the flask charged with  $\text{H}_2$  (1 atm.) The whole was then stirred at r.t. for 16 h. before being filtered through celite and then concentrated to afford the desired amine as a pale brown residue which was used in the next step without further purification (390 mg, quant. yield).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta_{\text{H}}$  2.65 (2H, d,  $J$  = 9.8 Hz,  $\text{CH}_2$ ), 1.40 (18H, s,  $\text{O}(\text{CH}_3)_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  85.7, 35.2, 34.7

### Di-tert-butyl (formamidomethyl)phosphonate **619**



Di-tert-butyl (aminomethyl)phosphonate **624** (490 mg, 2.21 mmol) was formylated according to general procedure 4 using acetic formic anhydride (192 mg, 1.70 mmol) and THF (5 mL) to afford the title compound as a colourless oil that was used without further purification (402 mg, 73% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 8.11 (1H, s, CHO), 6.96 (1H, br s, NH), 3.63 (2H, dd,  $J$  = 12.8, 6.0 Hz,  $\text{NCH}_2$ ), 1.49 (18H, d,  $J$  = 9.04 Hz,  $\text{O}(\text{CH}_3)_3$ ).

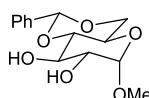
### Di-tert-butyl (isocyanomethyl)phosphonate **625**



Di-tert-butyl (formamidomethyl)phosphonate **619** (400 mg, 1.59 mmol) was dehydrated according to general procedure 2 when dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and  $\text{Et}_3\text{N}$  (1.95 mL, 14.34 mmol) and treated with  $\text{MsCl}$  (0.36 mL, 4.780 mmol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes :  $\text{EtOAc}$  (80 : 20),  $R_f$  0.28] to furnish title compound as a pale orange oil (101 mg, 22% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  3.59 (2H, d,  $J$  = 15.45 Hz,  $\text{CH}_2$ ), 1.51 (18H, s,  $\text{O}(\text{CH}_3)_3$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  159.0, 84.6, 40.5 (d,  $J$  = 158.0 Hz), 40.5, 30.2; IR (film  $\text{cm}^{-1}$ ):  $\nu$  = 2129 (N=C)

### Synthesis of Rhabduscin

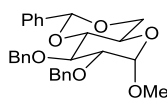
#### Methyl 4,6-*O*-benzylidene- $\alpha$ -D-glucopyranoside **634**<sup>218</sup>



Benzaldehyde dimethyl acetal (30 mL, 0.193 mol) was added to a solution of methyl- $\alpha$ -D-glucopyranoside **633** (25 g, 0.129 mol) and  $p\text{TSA}$  (30 mol%) in anhydrous DMF (250 mL) and the whole stirred for 2 h. at 60 °C under  $\text{N}_2$ . The reaction was neutralised by addition of  $\text{Et}_3\text{N}$ , and the solvents removed *via* distillation under reduced pressure. The resulting oil was partitioned between chloroform and water, and the organic phase dried over  $\text{MgSO}_4$  and concentrated (post filtration) to give a solid that was triturated with petroleum ether to afford the title compound as a white solid [petroleum ether:  $\text{EtOAc}$  (50 : 50),  $R_f$  0.01] (27.75 g, 76 % yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.53-7.46 (2H, m,  $\text{ArH}$ ), 7.40-7.34 (3H, m,  $\text{ArH}$ ), 5.53 (1H,

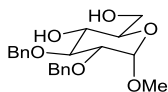
s, CHPh), 4.80 (1H, d,  $J = 3.8$  Hz, CH-1), 4.30 (1H, dd  $J = 10.1, 4.5$  Hz, CH<sub>2</sub>'), 3.93 (1H, app t,  $J = 9.8$  Hz, CH-3), 3.81 (1H, td,  $J = 9.7, 4.5$  Hz, CH-5), (3.75, app t,  $J = 9.7$  Hz, CH<sub>2</sub>'') 3.63 (1H, dd,  $J = 10.1, 3.8$  Hz, CH-2), 3.52 (1H, t,  $J = 10.1$  CH-4), 3.50 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_c = 137.1, 126.4, 128.4, 102.1, 99.9, 81.0, 71.9, 73.0, 69.0, 62.5, 55.7$ ; IR (powder, cm<sup>-1</sup>):  $\nu = 3415.41$  (O-H) 2937. 81 (C-H aromatic), HRMS (ESI) calculated for C<sub>14</sub>H<sub>19</sub>O<sub>6</sub> [M+H]<sup>+</sup>: found  $m/z$  283.1172, requires  $m/z$  283.1182.

### Methyl 2,3-di-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside **635**

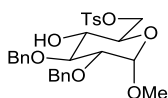


Benzyl bromide (44.2 mL, 0.372 mol) was added to a solution of Methyl 4,6-O-benzylidene-D-glucopyranoside **634** (15 g, 0.053 mol) and KOH (17.9 g, 0.319 mol) in anhydrous toluene. The resulting solution was refluxed under N<sub>2</sub> at 120 °C for 4 h., before being cooled to r.t. to allow for dilution with toluene and partition against water. The organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo* to afford the desired compound as a yellow oil which was used in the subsequent reaction without purification (21.13 g, 86% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H = 7.69$ -7.61 (4H, t,  $J = 8.76$  Hz, ArH), 7.56-7.29 (11H, m), 5.70 (1H, s, CHPh), 5.07 (1H, d,  $J = 11.3$  Hz, OCH<sub>2</sub>Ph), 5.00 (2H, dd,  $J = 12.3, 11.3$  Hz, OCH<sub>2</sub>Ph), 4.85 (1H, d,  $J = 12.3$  Hz, OCH<sub>2</sub>Ph), 4.75 (1H, d,  $J = 3.5$  Hz, CH-1), 4.71 (1H, s, CH), 4.63 (1H, s, CH), 4.42 (1H, dd,  $J = 5.2, 4.5$  Hz CH-3), 4.20 (1H, t,  $J = 9.3$  Hz, CH<sub>2</sub>'), 3.99 (1H, td,  $J = 10.0, 4.8$  Hz, CH-5), 3.85 (1H, t,  $J = 10.6$  Hz, CH<sub>2</sub>''), 3.75 (1H, t,  $J = 9.4$  Hz, CH-4), 3.71 (1H, dd,  $J = 10.6, 3.5$  Hz, CH-2), 3.55 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_c = 138.2, 127.6, 101.3, 99.2, 81.0, 79.2, 78.6, 75.3, 73.8, 69.1, 62.3, 55.4$ ; IR (film, cm<sup>-1</sup>):  $\nu = 3028.52$  (C-H aromatic); HRMS (ESI) calculated for C<sub>28</sub>H<sub>31</sub>O<sub>6</sub> [M+H]<sup>+</sup>: found  $m/z$  463.2093, requires  $m/z$  463.2121



**Methyl 2,3-di-O-benzyl- $\alpha$ -D-glucopyranoside 636<sup>219</sup>**

Iodine crystals (4.87 g, 0.0192 mol) were added to a solution of Methyl 2,3-di-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside **635** (24.0 g, 0.052 mol) in MeOH (500 mL) and refluxed under nitrogen at 80 °C for 16 h. The reaction mixture was allowed to cool to r.t. before aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was added and the solvents co-evaporated. The evaporation residue was partitioned between H<sub>2</sub>O and EtOAc and the organic phase was dried over MgSO<sub>4</sub>, concentrated under reduced pressure and purified using silica gel chromatography [petroleum ether : Et<sub>2</sub>O (50 : 50), R<sub>f</sub> 0.09] to afford the title compound as a yellow oil (17.5 g, 90 % yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  = 7.40-7.28 (10H, m, ArH), 5.02 (1H, d,  $J$  = 11.41 Hz, OCH<sub>2</sub>Ph), 4.74 (2H, dd,  $J$  = 11.97, 11.41 Hz, OCH<sub>2</sub>Ph), 4.66 (1H, d,  $J$  = 11.97 Hz, OCH<sub>2</sub>Ph), 4.60 (1H, d,  $J$  = 3.62 Hz, CH-1), 3.82-3.71 (3H, m CH-3 & OCH<sub>2</sub>), 3.61 (1H, dt,  $J$  = 9.8, 4.1 Hz, CH-5), 3.52 (1H, t,  $J$  = 9.6 Hz, CH-4), 3.49 (1H, dd,  $J$  = 9.6, 3.6 Hz, CH-2), 3.38 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  = 138.7, 137.9, 128.5, 128.5, 128.1, 128.0, 127.9, 127.9, 98.2, 81.2, 79.8, 73.1, 75.4, 70.71, 70.4, 62.4, 55.2; IR (film, cm<sup>-1</sup>):  $\nu$  = 3271.58 (O-H), 3060.49 (C-H aromatic); HRMS (ESI) calculated for C<sub>21</sub>H<sub>26</sub>NaO<sub>6</sub> [M+Na]<sup>+</sup>: found  $m/z$  397.1666, requires  $m/z$  397.1622.

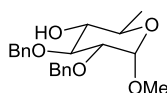
**Methyl 2,3-di-O-benzyl-6-O-p-toluenesulfonyl- $\alpha$ -D-glucopyranoside 637<sup>220</sup>**

A solution of Methyl 2,3-di-O-benzyl- $\alpha$ -D-glucopyranoside **636** (15.0 g, 0.04 mol) in pyridine (180 mL) under nitrogen was cooled to -20 °C, and tosyl chloride (11.45 g, 0.06 mol) added and the resulting solution was left to warm to r.t. After stirring for 16 h., the reaction mixture was heated at 60 °C for 30 min and then returned to r.t. and partitioned between aq. NaHCO<sub>3</sub> and CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was then dried using MgSO<sub>4</sub>, concentrated under reduced pressure and purified using silica gel chromatography [petroleum ether: EtOAc (50 : 50), R<sub>f</sub> 0.45] to afford the title compound as a pale yellow oil that solidified upon standing to yield a white

## Experimental

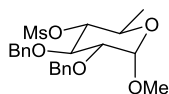
solid (15.56 g, 74% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.86 (2H, d,  $J$  = 8.34 Hz, ArH *tosyl*), 7.37-7.27 (12H, m, 10 ArH *benzyl* & 2 ArH *tosyl*), 4.99 (1H, d,  $J$  = 11.4 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.7 (1H, d,  $J$  = 11.8 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.70 (1H, d,  $J$  = 11.4 Hz  $\text{OCH}_2\text{Ph}$ ), 4.63 (1H, d,  $J$  = 11.8 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.56 (1H, d,  $J$  = 3.6 Hz *CH-1*), 4.23 (2H, d,  $J$  = 3.6 Hz,  $\text{OCH}_2$  *tosyl*), 3.67-3.69 (2H, m, *CH-3* & *CH-5*), 3.48-3.41 (2H, m, *CH-2* & *CH-4*), 3.32 (3H, s,  $\text{OCH}_3$ ), 2.43 (3H, s,  $\text{SCH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 144.8, 138.6, 137.9, 132.9, 129.8, 128.6, 128.5, 128.1, 128.0, 127.9, 127.9, 127.9, 98.1, 81.09, 79.5, 75.4, 73.2, 69.4, 55.3, 21.6; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 3503.03 (O-H), 2911.08 (C-H aromatic); HRMS (ESI) calculated for  $\text{C}_{28}\text{H}_{32}\text{NaO}_8\text{S}$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  551.1744, requires  $m/z$  551.1710.

### Methyl 2,3-Di-*O*-benzyl-6-deoxy- $\alpha$ -D-glucopyranoside **638**<sup>220</sup>



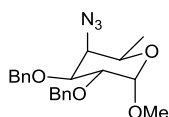
$\text{LiAlH}_4$  (0.65 g, 0.0170 mol) was added portion wise to a solution of Methyl 2,3-di-*O*-benzyl-6-*O*-*p*-toluenesulfonyl- $\alpha$ -D-glucopyranoside **367** (4.5 g, 0.0085 mol) in anhydrous THF. The resulting suspension was then refluxed (bath temp 80 °C) under nitrogen for 2 h. before being quenched by the careful addition of ice water. Once effervescence had ceased, the reaction liquor was filtered through celite (2 x 20 mL EtOAc washings), and the filtrate evaporation residue purified using silica gel chromatography to afford the title compound as a colourless oil (2.57g, 85% yield). [petroleum ether: EtOAc (50 : 50),  $R_f$  0.60];  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.40-7.28 (10H, m, ArH), 5.04 (1H, d,  $J$  = 11.3 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.76 (1H, d,  $J$  = 11.8 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.68 (2H, t,  $J$  = 12.4 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.57 (1H, d,  $J$  = 3.8 Hz, *CH-1*), 3.73, (1H, t,  $J$  = 9.4 Hz, *CH-3*), 3.65 (1H, dq,  $J$  = 9.0, 6.2 Hz *CH-5*), 3.52 (1H, dd,  $J$  = 9.51, 3.5 Hz, *CH-2*), 3.38 (3H, s,  $\text{OCH}_3$ ), 3.16 (1H, t,  $J$  = 9.5 Hz, *CH-4*), 1.21 (3H, d,  $J$  = 6.2 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 138.0, 138.0, 128.6, 128.5, 128.1, 128.01, 127.9, 127.9, 98.0, 81.3, 80.1, 75.3, 75.3, 73.0, 66.8, 55.1, 17.6; IR (film  $\text{cm}^{-1}$ ):  $\nu$  = 3362.69 (O-H), 3031.25 (C-H aromatic); HRMS (ESI) calculated for  $\text{C}_{21}\text{H}_{26}\text{NaO}_5$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  381.1708, requires  $m/z$  381.1678.

**Methyl 2,3-Di-*O*-benzyl-6-deoxy-4-*O*-methylsulfonyl- $\alpha$ -D-glucopyranoside 639<sup>221</sup>**



Methanesulfonylchloride (3.4 mL, 0.045 mol) was added to an ice cold solution of methyl 2,3-Di-*O*-benzyl-6-deoxy- $\alpha$ -D-glucopyranoside **638** (2.56 g, 0.007 mmol) and pyridine (5 mL) in  $\text{CH}_2\text{Cl}_2$  (15 mL). The solution was allowed to warm up to r.t. before being partitioned between  $\text{CH}_2\text{Cl}_2$  (50 mL) and aq.  $\text{NaHCO}_3$ , with the organics phase then being washed with brine and subsequently dried over  $\text{MgSO}_4$ . The evaporation residue from the organics was purified by silica gel chromatography [petroleum ether: EtOAc (50 : 50),  $R_f$  0.70], affording the title compound as a white solid (2.64 g, 87 % yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.36-7.27 (10H, m, ArH), 5.09 (1H, d,  $J$  = 11.1 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.74 (1H, d,  $J$  = 11.8 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.65 (2H, dd,  $J$ =11.8, 11.1 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.55 (1H, d,  $J$  = 3.8 Hz, CH-1), 4.20 (1H, t,  $J$  = 9.3 Hz, CH-4), 3.97 (1H, t,  $J$  = 9.6 Hz, CH-3). 3.99-3.82 (1H, dq,  $J$  = 9.3, 6.0 CH-5), 3.58 (1H, dd,  $J$  = 9.62, 3.87 Hz, CH-2), 3.83 (3H, s,  $\text{OCH}_3$ ), 2.79 (3H, s,  $\text{SCH}_3$ ), 1.33 (3H, d,  $J$  = 6.4 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 138.2, 137.6, 128.6, 128.5, 128.2, 128.2, 127.8, 127.6, 97.4, 83.2, 80.5, 75.4, 73.3, 65.3, 55.4, 38.8, 17.59; IR (powder,  $\text{cm}^{-1}$ ):  $\nu$  = 2979.79 (C-H aromatic); HRMS (ESI) calculated for  $\text{C}_{22}\text{H}_{28}\text{NaO}_7\text{S}$   $[\text{M}+\text{Na}]^+$ : found  $m/z$  459.1454, requires  $m/z$  459.1453.

**Methyl-4-azido-2,3-di-*O*-benzyl-4,6-dideoxy- $\alpha$ -D-galactopyranoside 640<sup>221</sup>**

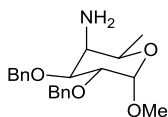


$\text{NaN}_3$  was added portion wise to a solution of Methyl 2,3-Di-*O*-benzyl-6-deoxy-4-*O*-methylsulfonyl- $\alpha$ -D-glucopyranoside **639** (2.00 g, 4.6 mmol), 15-crown-5 (0.318 mL, 1.61 mmol) in DMF (15 mL). The whole was stirred under nitrogen at 120 °C for 4 h. before being cooled to r.t. and the DMF removed *in vacuo*. The title compound was obtained as a yellow oil following purification using silica gel chromatography [petroleum ether: EtOAc (50 : 50),  $R_f$

## Experimental

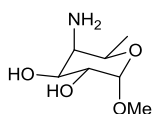
0.71] (1.01 g, 57 % yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.46-7.27 (10H, m ArH), 4.86 (2H, app. t,  $J$  = 11.3 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.77 (1H, d,  $J$  = 11.9 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.68 (1H, d,  $J$  = 11.9 Hz,  $\text{OCH}_2\text{Ph}$ ), 4.60 (1H, d,  $J$  = 3.6 Hz, CH-1), 4.06 (1H, dd,  $J$  = 9.88, 3.2 Hz, CH-3), 3.95- 3.85 (2H, m, CH-4 & CH-5), 3.71 (1H, d,  $J$  = 3.6 Hz CH-2), 3.36 (3H, s,  $\text{OCH}_3$ ), 1.25 (3H, d,  $J$  = 6.4 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 138.3, 128.5, 128.1, 127.9, 127.8, 127.7, 98.7, 78.1, 76.0, 73.7, 73.2, 65.0, 64.2, 55.4, 17.3; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 2903.87 (C-H aromatic), 2107.81 (N=N=N); HRMS (ESI) calculated for  $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_4$   $[\text{M}+\text{H}]^+$ : found  $m/z$  406.1744, requires  $m/z$  406.1743.

### 4-amino-2,3-di-*O*-benzyl- $\alpha$ -D-mannopyranose **641**



Methyl-4-azido-2,3-di-*O*-benzyl-4,6-dideoxy- $\alpha$ -D-galactopyranoside **640** (0.5 g, 0.0013 mol) was dissolved in a mixture of MeOH and aq. 1M HCl (1 : 1) (20 mL total) and Pd/C added (180 mg, 0.00013 mol). The resultant suspension was then stirred under an atmosphere of hydrogen for 24 h., before being filtered through celite and concentrated to afford the target compound as a pale yellow oil (crude).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 7.42-7.25 (10H, m, ArH), 4.84-4.67 (4H, app. m,  $\text{OCH}_2\text{Ph}$ ), 4.65 (1H, d,  $J$  = 3.8 Hz, CH-1), 4.02 (1H, q,  $J$  = 6.6 Hz, CH-5), 3.92 (1H, dd,  $J$  = 10.9, 4.1 Hz, CH-3), 3.84 (1H, dd,  $J$  = 10.9, 3.8 Hz, CH-2), 3.43 (1H, app br s, CH-4), 3.38 (3H, s,  $\text{OCH}_3$ ), 1.31 (3H, d,  $J$  = 6.6 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 138.4, 138.1, 128.3, 128.0, 127.9, 98.5, 75.0, 73.2, 72.6, 64.0, 55.3, 53.5, 16.6; HRMS (ESI) calculated for  $\text{C}_{21}\text{H}_{28}\text{NO}_4$   $[\text{M}+\text{H}]^+$ : found  $m/z$  358.2019, requires  $m/z$  358.2018

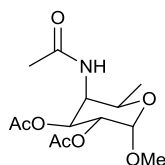
### 4-amino-2,3-dideoxy- $\alpha$ -D-mannopyranose **642**



## Experimental

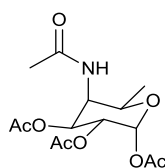
Methyl-4-azido-2,3-di-*O*-benzyl-4,6-dideoxy- $\alpha$ -D-galactopyranoside **640** (0.5 g, 0.0013 mol) was dissolved in a mixture of dioxane and water (1 : 1) (20 mL total) and Pd/C added (180 mg, 0.00013 mol). The resultant suspension was then stirred under an atmosphere of hydrogen for 24 h., before being filtered through celite and concentrated to afford the target compound as a pale yellow oil (30 mg - crude).  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta_{\text{H}}$  = 4.79 (1H, d,  $J$  = 4.4 Hz, CH-1), 4.13 (1H, q,  $J$  = 6.5 Hz, CH-5), 3.93 (1H, dd,  $J$  = 10.5, 4.4 Hz, CH-2), 3.77 (1H, dd,  $J$  = 10.5, 4.0 Hz, CH-3), 3.39 (3H, s,  $\text{OCH}_3$ ), 3.15 (1H, d,  $J$  = 4.0 Hz, CH-4), 1.23 (3H, d,  $J$  = 6.5 Hz,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (125 MHz,  $\text{D}_2\text{O}$ ):  $\delta_{\text{C}}$  = 99.7, 69.5, 67.9, 65.8, 55.6, 55.2, 16.4; HRMS (ESI) calculated for  $\text{C}_7\text{H}_{15}\text{NO}_4$   $[\text{M}+\text{H}]^+$ : found  $m/z$  178.1092, requires  $m/z$  178.1079.

### 4-*N*-acetyl-2,3-*O*-acetyl- $\alpha$ -D-mannopyranose **643**



The title compound was prepared according to general procedure 5 from 4-amino-2,3-dideoxy- $\alpha$ -D-mannopyranose **642** (50 mg, 0.31 mmol), DMAP (4 mg, 10 mol%) and  $\text{Ac}_2\text{O}$  (1 mL) in pyridine (5 mL). The title compound, a colourless glass, was used in the subsequent reactions without isolation. (30 mg approx.).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 5.58 (1H, d,  $J$  = 8.7 Hz, CH-1), 5.32-5.23 (1H, m, CH), 4.95-4.87 (1H, m, CH), 4.56-4.49 (1H, m, CH), 4.23 (1H, qd,  $J$  = 6.8, 1.5 Hz, CH-5), 3.39 (3H, s,  $\text{OCH}_3$ ), 2.11 (3H, s,  $\text{OAc}$ ), 2.08 (3H, s,  $\text{OAc}$ ), 2.00 (3H, s,  $\text{NAc}$ ), 1.16 (3H, d,  $J$  = 6.8 Hz).

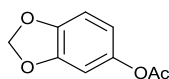
### 4-*N*-acetyl-1,2,3-*O*-acetyl- $\alpha$ -D-mannose **644**



Acetic anhydride (50  $\mu$ L) and  $\text{H}_2\text{SO}_4$  (4  $\mu$ L, 0.045 mmol) were added to a solution of 4-*N*-acetyl-2,3-*O*-acetyl- $\alpha$ -D-mannopyranose **643** (30 mg, 0.099 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL). The whole was allowed to stir at r.t. overnight before being quenched with aq.  $\text{NaHCO}_3$  and extracted with further  $\text{CH}_2\text{Cl}_2$ . The combined organics were dried over  $\text{MgSO}_4$  and the concentrated to afford the title compound as a dirty oil. (no yield)  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 6.23 (1H, d,  $J$  = 3.8 Hz, *CH*-1), 5.82 (1H, d,  $J$  = 9.4 Hz, *CH*-3), 5.20 (1H, dd,  $J$  = 7.6, 3.8 Hz, *CH*-2), 4.56-4.28 (1H, m, *CH*-4), 4.29 (1H, qd,  $J$  = 6.4, 1.5 Hz, *CH*-5), 2.08 (3H, s, OAc), 2.01 (3H, s, OAc), 1.95 (3H, s, OAc), 1.94 (3H, s, NAc), 1.10 (3H, d,  $J$  = 6.8 Hz).

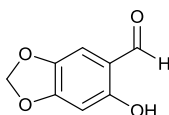
## 6.5 Compounds associated with the synthesis of Paerucumarin (Chapter 5)

### 5-hydroxybenzo[1,3]dioxole acetate **705**<sup>204</sup>



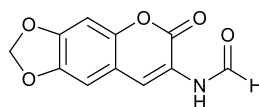
The title compound was prepared according to general procedure 5 from sesamol **704** (5.00 g, 0.036 mol), DMAP (0.40 g, 10 mol%) and  $\text{Ac}_2\text{O}$  (10 mL) in pyridine (30 mL). The title compound, a colourless oil, was used in the subsequent reactions without the need for purification (5.78 g, 89% yield).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  = 6.63 (1H, d,  $J$  = 8.3 Hz, *ArH*), 6.48 (1H, d,  $J$  = 2.3 Hz, *ArH*), 6.39 (1H, dd,  $J$  = 8.3, 2.3 Hz), 5.79 (2H, s,  $\text{OCH}_2\text{O}$ ), 2.10 (3H, s, OAc);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  = 169.7, 148.0, 145.3, 145.0, 113.9, 107.9, 103.7, 101.7, 20.8; IR (film,  $\text{cm}^{-1}$ ):  $\nu$  = 2903.69 (C-H aromatic), 1757.08 (C=O).

### 6-Hydroxybenzo[1,3]dioxole-5-carboxaldehyde **706**<sup>204</sup>

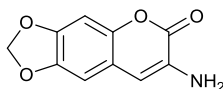


5-hydroxybenzo[1,3]dioxole acetate **705** (5.68 g, 0.031 mol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and cooled to 0 °C before SnCl<sub>4</sub> (9.77 mL, 0.063 mol) and then Cl<sub>2</sub>CHOCH<sub>3</sub> (3.42 mL, 0.037 mol) were added sequentially through a dropping funnel. After two hours of stirring the reaction mixture was poured over ice and the resultant organic phase washed with aq. 2M HCl (3x 20 mL) and H<sub>2</sub>O (2x 20 mL) before being dried over MgSO<sub>4</sub> and concentrated (post filtration) to afford a crude solid. Purification was achieved upon trituration with cold MeOH to yield the desired product as a white powder (4.21 g, 81% yield): m.p. 123-125 °C (reference: 125-126 °C); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 9.62 (1H, s, CHO), 6.86 (1H, s, ArH), 6.47 (1H, s, ArH), 6.02 (2H, s, OCH<sub>2</sub>O); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 193.7, 161.5, 141.34, 113.6, 109.3, 102.1, 98.3; IR (film, cm<sup>-1</sup>): ν = 2983.00 (C-H aromatic), 1641.49 (C=O).

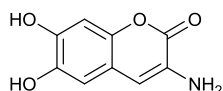
***N*-(6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-7-yl)formamide 707**



CuI (22 mg, 0.12 mmol) was added to a stirring mixture of 6-hydroxybenzo[1,3]dioxole-5-carboxaldehyde **706** (200 mg, 1.20 mmol), isocyanoacetate (0.135 mL, 1.20 mmol) and pyridine (0.1 mL, 1.20 mmol) in MeOH (5 mL) at 50 °C. After stirring for 10 h., the reaction was cooled to 0 °C and the precipitated product collected by filtration. Further washing with cold MeOH (2 mL) afforded the title compound as a pale pink solid (222 mg, 58% yield). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ<sub>H</sub> = 10.12 (1H, br s, NH), 8.59 (1H, s, CHO), 8.37 (1H, s, C=CH), 7.30 (1H, s, ArH), 7.13 (1H, s, ArH), 6.14 (2H, s, OCH<sub>2</sub>O); <sup>13</sup>C NMR (75 MHz, DMSO): δ<sub>C</sub> = 161.5, 157.83, 149.6, 146.6, 125.1, 121.8, 113.3, 105.6, 102.7, 98.0; IR (film, cm<sup>-1</sup>): ν = 3335.82 (N-H), 3044.73 (C-H aromatic), 2915.49 (C-H aromatic), 1713.41 (C=O ester) 1676.65 (C=O formamide); HRMS (ESI) calculated for C<sub>11</sub>H<sub>8</sub>NO<sub>5</sub> [M+H]<sup>+</sup>: found *m/z* 234.0394, requires *m/z* 234.0402.

**7-amino-6H-[1,3]dioxolo[4,5-g]chromen-6-one 709**

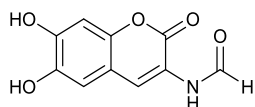
*N*-(6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-7-yl)formamide **707** (200 mg, 0.862 mmol) was refluxed in a 10 mL mixture of MeOH and 33% HCl (8 : 2) for 1 h. After this time, the reaction mixture was cooled and neutralised to pH 8 using K<sub>2</sub>CO<sub>3</sub>, filtered and then concentrated to afford the target compound as a bright red solid, which did not need purification (174 mg, nr quant. yield). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ<sub>H</sub> = 7.23 (1H, s, *ArH*), 7.12 (1H, s, *ArH*), 7.05 (1H, s, *ArH*), 6.08 (2H, s, CH<sub>2</sub>), 5.58 (2H, br s, NH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ<sub>C</sub> = 158.7, 148.3, 146.0, 145.0, 126.0, 119.8, 114.2, 104.4, 102.4, 98.0; IR (film, cm<sup>-1</sup>): ν = 3435.79 (N-H), 1718.01 (C=O); HRMS (ESI) calculated for C<sub>10</sub>H<sub>7</sub>NO<sub>4</sub> [M+H]<sup>+</sup>: found *m/z* 206.0440, requires *m/z* 206.0453.

**3-amino-6,7-dihydroxy-2H-chromen-2-one 710**

BBr<sub>3</sub> (150 mg, 0.731 mmol) was added to a suspension of 7-amino-6H-[1,3]dioxolo[4,5-g]chromen-6-one **709** in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) held at -78 °C under an N<sub>2</sub> atmosphere. After stirring for 4 h. the reaction was quenched with methanol and the target compound, a red solid, was collected by Buchner filtration and needed no further purification (141 mg, nr quant. yield). <sup>1</sup>H NMR (250 MHz, DMSO-d<sub>6</sub>): δ<sub>H</sub> = 7.52 (1H, s, *ArH*), 6.99 (1H, s, *ArH*), 6.81 (1H, s, *ArH*), 6.09 (2H, br s, NH<sub>2</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ<sub>C</sub> = 161.47, 159.67, 148.92, 146.62, 143.06, 132.66, 115.33, 112.39, 105.73; IR (film, cm<sup>-1</sup>): ν = 3705.36 (O-H), 3505.36 (N-H), 1711.91 (C=O); HRMS (ESI) calculated for C<sub>9</sub>H<sub>8</sub>NO<sub>4</sub> [M+H]<sup>+</sup>: found *m/z* 194.0451, requires *m/z* 194.0453.



## Pseudoverdin 700<sup>201</sup>



3-amino-6,7-dihydroxy-2H-chromen-2-one **710** (120 mg, 0.622 mmol) was formylated by refluxing in formic acid (20 mL) for 4 h., the title compound being collected as a beige solid upon Buchner filtration (141 mg, 96% yield). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ<sub>H</sub> = 10.0 (1H, s, NH), 8.45 (1H, s, C=CH), 8.35 (1H, s, CHO), 6.95 (1H, s, ArH), 6.75 (1H, s, ArH); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ<sub>C</sub> = 161.0, 157.8, 148.0, 144.5, 143.5, 125.3, 120.5, 111.9, 110.7, 102.6; HRMS (ESI) calculated for C<sub>10</sub>H<sub>7</sub>NO<sub>5</sub> [M+H]<sup>+</sup>: found *m/z* 220.0235, requires *m/z* 220.0245

## 6.6 Biological Procedures/Investigations

### Identifying hit compounds using disk diffusion assays

The majority of the susceptibility testing of bacteria using disk diffusion were carried out by Liam Stephens, whilst phenol vinyl-isocyanide **542** and Byelyankacin **544** were investigated under the direction of Diana Alves.

A cotton-bud that had been dipped in a pre-prepared inoculum of bacteria was swabbed/streaked over the entire surface of a Mueller-Hinton agar petri-dish, with subsequent rotations of the disk (60 °) ensuring even coverage. Meanwhile, dispense disks are soaked for 15 min. in a 1mg ml<sup>-1</sup> solution of the antibiotic dissolved in a 9 : 1 mixture of water and methanol. Once the compound has been loaded onto the disk, the disk is carefully placed on the surface of the agar and the dishes incubated at 37 °C for 16 hours in ambient air. After this time the diameter of the 'clear' bacteria-free zone was measured using a ruler.

## Quantifying hit compounds using MIC

All antibiotic minimum inhibitory concentrations (MIC) of the antibiotic compounds were determined by Liam Stephens.

96-well microplates, each containing a volume of 200  $\mu$ l with 1 : 2 dilution of the antibiotic (64 to 0.0625  $\mu$ g/ml range) were inoculated with an initial standard inoculum of a 105 cfu/ml of an overnight culture. A 0.5 McFarland scale was used for inoculum standardisation. Incubation at 37 °C with shaking (90 rpm) followed for 18 hours. The MIC for each isolate was scored by direct visualisation. A negative control containing only broth was added and the experiments were run in triplicate.

## 6.7 Computation calculations using Spartan

The conformational energies of phenol vinyl-isocyanide **542** was run using Spartan'14 student edition (trial version) under the direction of Prof. Ian Williams.

The geometry of each isomer was fully optimised to a planar structure in an energy minimum with all real vibrational frequencies. Free energy corrections at 298.15 K and 1 atm are calculated using ideal gas, rigid-rotor and harmonic oscillator approximations and include zero-point energy and thermal partition-function contributions. Both the EDF2 and B3LYP density functionals have been employed with the 6-31G\* basis for optimisations and vibrational frequencies and the 6-311+G\*\* basis set for geometries only. Most of the energy difference between the isomers came from the free-energy correction (zero-point energy, thermal energy and entropy) rather than from their intrinsic potential energies. At 25 °C an equilibrium mixture contains between 75% and 81% of the more stable *trans* isomer.

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